

nature

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Messages from the minefield

To attempt to comment on salary levels of scientists is to venture into a minefield. Off in various directions are snares such as historical precedents, parities between government, universities and industry, relations to the cost of living index, and rivalries, even jealousies, between different professions. The appearance of the latest remuneration survey of the Institute of Physics (*Physics Bulletin*, April 1976), however, presents a good opportunity to review the way that physicists are paid. With severe and externally imposed restraint on increases in income this year, and probably next, now is also a good time to look at a scene relatively uncluttered by salary negotiations.

The Institute of Physics has carried out salary surveys for more than 20 years, and although there has been considerable variability in the method of reporting, it is possible to see general trends. The table shows the median salary for Fellows of the Institute (the top grade; roughly 20% of the total membership). Where this figure has not

theless, two points can be made: that the rewards of being a scientist grew steadily until the mid-1960s, and that since then the rewards have barely held their own.

For the past year or two we have all been rediscovering industry, relearning that the country cannot function without a productive industrial sector, attempting to help industry by increasing university links, and so on. But figures from the survey show how unattractive industrial salaries look in comparison with government and university pay. In 1974 the median salaries for all the institute's membership (not just Fellows) were £3,400 in industry, £4,420 in university, £3,500 in central government. By 1976 industry and university medians had moved roughly with the cost of living to £5,000 and £6,300, while central government had risen all of 70% to £6,000.

A cross-check on these last figures can be found in the most recent remuneration survey of the Institute of Electrical Engineers for January 1976. This confirms that

Date of survey	1951	1953	1956	1960	1964	1967	1971	1974	1976
Median salary of Fellows (£)	1,400	1,550	1,950	2,350	3,300	3,600	4,620	5,850	8,260
Median in 1974 (£)	3,800	3,900	4,300	4,800	6,000	5,800	6,000	5,850	5,550

been explicitly published, an attempt has been made to extract it from the statistics available. The median for the top grade may not be the ideal pointer, but it is the only one easily available, and nothing seen in other data gives the impression that it is out of line with other trends.

The table also contains salaries converted to January 1974 levels by use of the cost of living index. Purists will no doubt shudder at the misuse of statistics: no allowance has been made for the progressive nature of income tax, and the cost of living index is only a helpful guide to those whose salaries do not differ too much from the national average. As a result the contrast between the 1950s and the 1970s is probably not as marked as it seems. Never-

median salaries in central government, the nationalised industries and universities are more than £1,000 higher than those in industry. No doubt there can be bickering over fringe benefits, security of tenure, relative qualifications, pension schemes, and so on, but the message is clear enough: the sector of the economy that is looked upon to solve our present economic woes cannot match the salary rewards that are available elsewhere; and with pay restraint this balance is unlikely to be redressed for some years. Historically, salaries in the three spheres of government, university, and industry have never got so far out of line as this. Does the government realise the consequences, including that of damping mobility out of the public sector?

Concorde's circadian conquest

"BUSINESSMEN interviewed as they disembarked at Heathrow Airport recently after a British Airways Concorde flight from Bahrain had one thing in common—they felt alert without any suggestion of 'jet lag'."

So runs a recent British Airways advertisement for Transatlantic Concorde. The operation is about as meaningful as being assured by the departing clientele of a pub at closing time that they are not suffering from a hangover.

There is no precise definition of what jet lag is, but if you gave those businessmen at Heathrow time to think for a bit they would probably perceive through the haze of Chateau Brane Cantenac '70 that they couldn't know whether they were jet lagged until they had found out whether their internal clocks were waking them up at the witching hour of 3 AM the following morning. And that

will happen, Concorde or 707. But no doubt in their haste to get back to their office they would settle for a definition of jet lag as the way they felt on emerging from the plane. Besides, you've got to justify the elevated price by something besides the smoked salmon and peaches in kirsch.

Now all this is as harmless as claiming that a certain dogfood prolongs active life or a certain sauce is 99.46% pure. But wait until the businessmen are being enticed to fly to a day's work in New York and to return to London the same day. British Airways won't be interviewing them at Heathrow about jet lag, because they'll be travel-weary. They'll be rung up the following morning in their office and asked did they wake at an unearthly hour. Of course they didn't—Concorde cuts jet lag either way. □



Coal comfort for Britain?

Allan Piper reports on the fortunes of the British coal industry, reflected in the recent controversial decision to develop the Selby coalfield

PERHAPS the most important point to be made about the plans to open up the Selby coalfield is that just three years ago any idea that the National Coal Board (NCB) might invest up to £400 millions in a single new venture would probably have been dismissed as economically senseless. In steady decline since the 1950s, the industry seemed by 1973 to be plunging inexorably to extinction, pulled by the ever tightening purse strings of successive governments intractably committed to oil and nuclear power by the strategy laid down in a 1967 White Paper on Fuel Policy. But the hiccup that arrived with the Arab oil crisis forced another look at the shape of coal's future—and the Selby project has become, perhaps deceptively, the banner heading coal's march back to prominence in Britain's energy strategy.

If all goes according to plan, the first Selby coal will arrive at the surface within four years, and by 1985 the new field should provide 10 million tons annually. But while the Selby scheme is ambitious enough itself, its real significance lies in its role as part of a wider "Plan for Coal" developed to boost sagging production levels over the next ten years. Quick to realise the opportunities presented by the fuel price adjustments of recent years, the NCB along with government and union officials formulated the ten year plan back in 1974 in an effort to stabilise and expand the British coal industry.

The aim is to introduce 42 million tons of new capacity by 1985, thus outstripping the decline of exhausted reserves and boosting production from last year's 125 million tons to an annual level of 150 million tons. Though almost half, and possibly more, of the new capacity is expected from new mines such as those on the Selby field, an equally significant amount will be brought out from pits now scheduled for extension and modernisation. And with exploration programmes proving around 500 million tons of new workable deposits each year (some even as far south as the edge of the London Basin), supplies look safe for at least another century at existing extraction rates.

In support of the expansion drive, the NCB has already begun stepping up its programme to introduce new mining equipment and techniques, and mining research is now firmly focused on the

development of remotely controlled underground machinery. Initial estimates put the necessary research and development expenditure at £33 millions up to 1980. The increase in mechanisation will not see a reduction in the existing work force: the NCB view is that recruitment must average around 28,000 a year—8,000 a year more than during the cutbacks of the last decade—just to stabilise output levels. By the time target levels are achieved in the mid-1980s the industry should maintain a stable work-force of 250,000, with a hoped-for productivity growth in excess of 4% a year.

The massive investment programme at the heart of the ten year plan was put at an estimated £1,400 millions at 1974 prices, a figure which included £600 millions in new investment. These figures are already being revised to £1,800–£2,000 millions and £1,000 millions respectively because of inflation, and the NCB might even be looking for special subsidies in the future if it cannot raise the money itself. Significantly, though, the coal industry was one of the few areas to escape the heavy spending cuts announced in a recent White Paper on Public Expenditure; NCB borrowing powers have been extended from £700 millions to £1,100 millions under the National Coal Board (Finance) Act, and a further increase to £1,400 millions awaits House of Commons approval. The NCB has also received strong European support through its membership of the European Coal and Steel Community (ECSC). Since Britain joined the EEC in 1973, the Community has approved loans for the NCB totalling £143 millions.

While the new flow of cash is crucial to the revitalisation of the industry, the UK Energy Secretary, Mr Anthony Wedgwood Benn, has not been unmindful of the need for comfortable union-management relationships. He admitted that union thinking influenced his recent decision to reappoint Sir Derek Ezra as NCB Chairman when his present term ends in July, and actually published the text of a letter seeking union advice on new appointments. Ezra is regarded favourably by the unions within the industry; his period of office has so far spanned two major national coal strikes, but he has managed to remain on consistently good terms with them. Last year a deficit



Photo: WHO

Coal face

of £112 millions was turned round to a profit of £34 millions, and last week Ezra gave estimates that the NCB broke even in 1975–76. His new term will run to 1979, by which time the ten year plan will be well under way.

All of which seems encouraging enough. Certainly with such key issues as finance and industrial relations apparently resolved, the coal industry is anticipating a rosy future, with coal occupying a central role in any long term energy strategy. The large demands of the British steel industry are expected to rise with the next economic upturn, and recent progress in the development of high-grade coking coal may curb future demands for imports. Even more important to the NCB is the judgment that by the mid-1980s nuclear and natural gas resources can between them fulfill considerably less than half of Britain's annual energy requirements of between 400 and 500 million tons coal equivalent. And while much of the shortfall, which could be as high as 370 million tons coal equivalent, will be met by indigenous and imported oil, competitive coal prices, the NCB argues, should ensure demands for its entire output, particularly as cheap imported coal is expected to have become unavailable.

But whether or not that prognosis holds true hinges largely upon the NCB's largest single customer, the Central Electricity Generating Board (CEGB), which last year burned 67 million tons of NCB coal in its power stations (alongside which the South of Scotland Electricity Board consumed a further 4 million tons). And here the critical problem arises. The CEGB is showing a marked reluctance to commit itself to coal-fuelled generating power while oil, gas and nuclear options remain available. One reason seems to

be concern at the NCB's record of efficiency. But another is escalating price rises. The NCB, as a member of the ECSC, remains unfettered by national pricing policy, and over the past two years coal prices have rocketed by an alarming 170%; the latest 15% increase adds an estimated £150 millions to the CEBG fuel bill, pushing it up to a colossal £1,600 millions a year. The CEBG chairman, Arthur Hawkins, was sharply critical: "The coal board", he said at a February meeting with Ezra, "might at least have invited its biggest customer to discuss at the highest level the serious implications of the increase, not only for electricity but for coal's prospects in the power station fuel market".

Although taking the point, Ezra's difficulty is that his hands are virtually tied. Confronted with powerful unions, the NCB finds almost half of its production costs are wage bills, while over half of present annual production is won unprofitably from older pits, using outdated machinery and production methods. The increasing volume of reserve stocks piling up at pitheads and power stations around the country adds to the problem: reserves are now standing at 30 million tons, worth £500 millions in realisable income, and costing the coal board £3 a ton in interest on borrowed capital each year. Last year excess sales over running production reduced the stockpiles by less than 10%, but it is a measure of the severity of the situation that before the NCB borrowing ceiling was raised last month, requirements rose above the earlier limit by £75 millions, an excess that had to be provided from government contingency funds.

Success for the NCB plan, then, is to an important extent contingent upon CEBG agreement to convert oil and gas-fired power stations to coal. In proposing an 11 million ton increase in coal burning by the CEBG over the next two years, Ezra has pointed out that a switch from oil and gas to coal could save the electricity industry £3 to £4 on each ton of fuel, and the country £250 millions on the balance of payments. The idea reportedly has the backing of the Department of Energy, but Hawkins has dismissed it as "quite ridiculous" at a time of expenditure cutbacks in the nationalised industries and falling consumer demand (which last year dropped by an estimated 5%). Further, aside from conversion costs, Hawkins has also to consider the heavy national investment in North Sea oil and gas.

As for the less immediate outlook, NCB hopes are similarly overshadowed by CEBG reluctance to order new coal-fuelled power plant. Though the coal-fired, 2,000 MW installation at Drax in

Yorkshire has just reached completion, 5 of the 12 remaining installations still under construction in Britain are nuclear powered, and 6 are oil-fired; only the 1,500 MW Aberthaw B in South Wales, due on-line around the end of the decade, is designed to burn coal. In addition, CEBG last month announced that forward ordering plans, already unlikely to favour the NCB, may well be revised downwards following the most recent demand forecasts. These indicate that consumer demand for the winter of 1982-83 will not rise above a peak of 52,000 MW, a surprising 2,000 MW down on last year's forecast for 1981-82, and only around 25%—or 3.5% a year—over current demands.

The CEBG has nonetheless made a couple of moves towards raising its requirement for NCB coal. Imports, which last year totalled 4.5 million tons, are to be cut. Hawkins recently announced that import contracts undertaken during 1973-74 to offset the then prevailing shortage of British reserves are to be run down over the next financial year. And CEBG demand for British coal last year topped the previous year's level by 3 million tons, while oil consumption was cut by almost a third to only 14 million tons coal equivalent. This turn-round indicates that if the price, quality and demand is right, the CEBG's 111 coal-fired installations could burn more coal than at present—in fact the requirement could rise as high as 90 million tons of coal a year, a figure more compatible with the aims of the ten year plan than the existing level of 67 million tons. The present shortfall occurs simply because demand levels above the early morning baseline of 22,000 MW are now met by oil-fired generating power in preference to coal power. But once the Plan for Coal gathers sufficient momentum, increasing productivity and cutting production costs, the NCB could swing that method of priority operation to its own advantage.

tage, leapfrogging coal-fired generating power above the oil and nuclear options.

Should that possibility not prove successful, export markets offer little prospect of an easy solution to the glut. In north-western Europe, the only realistic potential outlet, British coal will face competition not only from low-priced North American exports, but also from Polish coal maintained at continually depressed "administered prices" which undercut competition.

The £400 millions Selby project, however, in keeping with the optimistic front it presents to the outside world, remains for the most part unscathed by the wider complications, though critics of the ten year plan may disagree. The Main Barnsley Seam, stretching for 110 square miles beneath the Vale of York and an amenable three or so metres thick, will provide almost half of the new annual output by the mid-1980s. Geological conditions are sufficiently favourable to allow extraction using "drift mining" techniques, involving production through a single sloping tunnel rather than multiple vertical shafts. Production is expected to begin within four years, and when full operational capacity is achieved by the mid-1980s a maximum of 52,000 tons of coal a day will travel by conveyor to the surface, providing the nearby coal-fired power stations at Drax, Eggborough and Ferrybridge with continuous fuel supplies by rail.

Ezra says Selby will produce "the coal which, with North Sea oil and gas, is going to make Britain the only main nation in Europe which will be self-sufficient in energy by the mid-1980s". With the NCB also engaged on large research programmes in pyrolysis and liquefaction techniques, and British and European research efforts continuing in the areas of fluidised bed combustion and coal gasification, the future does seem promising. But there are still uncertainties to be removed if the turn-round is to materialise. □



Coal cutting machine

USA

The threat to peer review

Colin Norman reports from Washington on a series of events which provides a vivid example of the difficulties sometimes encountered by scientists engaged in research on controversial topics

IN A move believed to be without direct precedent, the House of Representatives on April 13 voted to shut off funds for a scientific research project because a powerful Congressman believes the project is scientifically unsound and morally unacceptable. A number of scientists have charged that the vote, if upheld in the Senate, constitutes a worrying challenge to academic freedom.

The project is certainly controversial. An attempt to determine the effects, if any, of marijuana on human sexual responses, it attracted considerable press attention when it was approved last year, and it has stirred up various expressions of moral outrage. Nevertheless, the research has been judged scientifically meritorious by committees of scientists expert in the field, endorsed by the federal government's top advisory committee on drug abuse, and been subjected to a searching inquiry by the government's senior health officials. If Congress does abort the project, the move is certain to be interpreted in the scientific community as political interference with the painstaking peer review process used by virtually every government agency to determine the relative merits of research proposals.

The project is a two-year research effort to be conducted by Dr Harris Rubin, a behavioural scientist at Southern Illinois University Medical School, Carbondale. Rubin's research protocol notes that though there have been many verbal and subjective reports indicating that marijuana has a substantial effect on human sexual behaviour, those effects and the drug's mode of action have not been objectively evaluated. His project involves giving measured doses of marijuana to volunteers and then measuring their physical responses to visual stimuli such as erotic films. Part of the experiment also involves monitoring blood levels of various sex hormones, such as testosterone.

Rubin applied for a grant from NIH for his project in May 1974, and his application was passed on to the National Institute on Drug Abuse (NIDA). It was reviewed for scientific merit by a peer review committee in

the usual manner, and two well known behavioural scientists — Dr Joseph Brady of Johns Hopkins University and Dr Jonathan Cole of Columbia University—conducted a site visit and discussed the application with Rubin in January 1975. The peer review committee subsequently approved the project as being scientifically valid, and NIDA decided to fund it in June 1975 with a grant of about \$121,000 over two years.

It was then that Rubin's troubles began. A routine announcement of the grant by Southern Illinois University was picked up by the press, several accounts of the project were published, and it was attacked in the *St Louis Globe Democrat* as "tax-paid debauchery". Then Senator William Proxmire, the colourful Senator from Wisconsin who has frequently denounced scientific research which he considers trivial or unsound, took the project in his sights and fired off a press release calling it "one of the most shocking examples of the 'federal love machine' I have ever found". That attracted considerable attention from the media.

While that furore was beginning to break, Rubin applied to the Department of Justice for a grant of immunity from federal prosecution, normally a routine procedure for scientists conducting approved studies with illicit drugs. The application turned out to be far from routine, however, for the Justice department sat on his application for six months, and in January this year it asked the Secretary of the Department of Health, Education and Welfare (of which NIDA is a part) to reevaluate the project and determine whether Rubin is suitably qualified to conduct the research. According to HEW officials, the Justice Department's action is unprecedented.

The reevaluation was carried out on March 11 at a closed meeting attended by the head of the Food and Drug Administration, the Assistant Secretary for Health, the head of NIDA, other government officials and a number of outside scientists—probably the most high-powered committee ever to consider a single grant application. According to HEW sources, the meeting resulted in a recommendation to David Matthews, Secretary of HEW, that the project continue to be supported. Matthews has not yet acted on the recommendation. Similarly, on February 11, the NIDA National Advisory Council, a committee composed of scientists and lay members, unanimously passed a resolution urging

Secretary Matthews to support the project.

Rubin has received nearly half of the grant from NIDA, but his project is in limbo until he receives his grant of immunity from the Justice Department.

Meanwhile, Congress entered the picture largely through the work of Representative Robert Michel, the House Republican Whip, who represents Peoria, Illinois. Michel, a powerful member of Congress, is the most senior Republican on the appropriations subcommittee which handles HEW's budget. He has denounced Rubin's project on several occasions, calling it "offensive to the sensibilities of most Americans", and he has twice quizzed HEW officials about it.

Michel made his decisive move against the project by writing into a supplemental appropriations bill containing funds for a number of different agencies, a provision cutting off support for Rubin's research. The bill was passed by the House on April 13 with scarcely anybody noticing Michel's handiwork. The bill is now being considered in the Senate, and a number of scientists have already written to their Senators to ensure that the provision doesn't slip past that body unnoticed.

Though there may indeed be questions about the utility of Rubin's research proposal, the fact is that the project has been endorsed by Rubin's scientific peers, and it has been approved after a painstaking review procedure which is a basic ingredient of federal support for science. If Congress were to overturn the funding decision, it would be an unprecedented step. Dr Joseph Brady, one of the chief reviewers of the project, last week called the House vote "outrageous", and pointed out that the review procedure "was a model of the way the peer review system should and can work".

Dr Robert DuPont, the head of NIDA, noted in a telephone interview last week that "Congress has the perfect right to assert itself in terms of how federal money is spent", but he added that "if Congress is going to reverse the considered judgment of the peer review process, I think it should be extremely cautious about setting a precedent".

As for Rubin, he said that he is "very disappointed" by the House vote, and he argued in a letter sent to several of his colleagues that if the Senate follows suit, "the Congress will be overruling the peer review process and politicians rather than scientists will be determining what specific research projects will be conducted . . . the danger to scientific and academic freedom in this precedent is enormous". □

USA

AFTER several months of negotiations richly laced with minor squabbles, key Senators and Congressmen have at last reached agreement on a bill to reestablish a science policy office in the White House. Final Congressional approval of the measure is generally deemed imminent, and the office will probably be off and running by the end of May—nearly three and a half years after Mr Nixon, arguing that he no longer required the services of a full-time science adviser, dismantled the former White House office of Science and Technology.

The bill has been in limbo for weeks while Congressional staff members and their bosses have been trying to work out a compromise between three conflicting versions of the legislation—a modest proposal put forward last June by President Ford, a slightly more ambitious measure approved in November by the House and a more substantial bill passed by the Senate in February. A compromise was finally struck in mid-April, and its swift approval by the House and the Senate is virtually assured.

It would establish a small office of Science and Technology Policy (OSTP) in the White House, headed by a director who would also double as the President's Science Adviser, and four associate directors.

Though it has always been assumed that the office would provide advice and assistance to the President and other White House bodies on matters involving science and technology, its exact role and scope have been in dispute. The compromise version of the bill specifies that the Director of OSTP will be a member of the Domestic Council, a top-level White House policy-making body for domestic affairs, and he will also be a statutory adviser to the National Security Council, the powerful defence and foreign policy committee formerly headed by Henry Kissinger. Those roles will at least ensure that the office will have a voice in both civilian and defence policy.

As for budgetary matters, the bill simply requires OSTP to assist the Office of Management and Budget in preparing the Administration's annual budget request, a role which falls far short of the powerful voice in fiscal matters for which many scientists had hoped. The bill does, however, provide two other mechanisms for OSTP to influence budgetary policies. First, it requires the office to prepare a five-year forecast of emerging national problems and provide a set of programme options to every government science agency at the

start of each budget cycle. Secondly, it requires OSTP to publish an annual report on federal science and technology, a mechanism which should allow the office to make public its views on the budgetary state of science.

Another important provision in the bill will create a President's Committee on Science and Technology,



consisting of between 8 and 14 prominent people, to conduct a two-year survey of the entire federal science and technology enterprise. The President will have the option of making the committee into a permanent body when it has completed its study.

Though the office has yet to be formally established, there is considerable speculation about who President Ford will choose to head OSTP. According to Congressional and Administration sources, there are three potential candidates: Dr Simon Ramo, Vice President of TRW Inc., Dr William O. Baker, President of Bell Labs, and Dr H. Guyford Stever, Director of the National Science Foundation, who now doubles as the President's part-time science adviser.

● The Nuclear Regulatory Commission (NRC) has, as expected, denied a petition seeking a drastic tightening of federal standards governing permissible levels of exposure to plutonium. The petition, filed in February 1974 by the Natural Resources Defense Council (NRDC), was based on the so-called "hot particle" theory which suggests that tiny particles of inhaled plutonium pose a severe health hazard because they lodge in the lung and deliver a prolonged, intense dose of radioactivity to the surrounding tissue. Since present plutonium exposure standards are based on the average dose of radioactivity to the whole lung, rather than to tissue around inhaled particles, the NRDC petition argued that the standards are too lax and should be tightened by a factor of 115,000. The matter has been a subject of considerable debate.

The NRC turned the petition down, and denied NRDC's request for public hearings on the matter, however, because it found that "scientific evidence does not support the technical position upon which the NRDC petition is based". Drawing upon several studies, including reports by the UK Medical Research Council and the former Atomic Energy Commission, the NRC believes that the hot particle theory is flawed in several respects and does not provide a valid basis for establishing exposure standards.

NRDC has also petitioned the Environmental Protection Agency (EPA) with a similar request. EPA is awaiting a report on the hot particle theory from the National Academy of Sciences (expected in about a month) before issuing its reply.

● Following much prodding from Congress and a good deal of agitation from a number of independent expert groups, the Energy Research and Development Administration (ERDA) last week placed increased emphasis on energy conservation in its overall plan for decreasing the United States' dependence on foreign sources of oil. The new emphasis is incorporated in an updated plan for energy research and development, published last week by ERDA, which spells out in some detail the agency's goals and strategies.

The plan, an expanded and revamped version of a document published by ERDA on June 30 last year, ranks the development of technologies to increase energy efficiency and conservation among ERDA's highest priorities. Unveiling the plan at a press conference, Dr Robert C. Seamans, Jr, ERDA's Administrator, said he believed it was "impossible to exaggerate the need and desirability to make more efficient use of energy".

Though the plan isn't very specific about the exact strategies envisaged for conservation, Seamans said that the agency would be putting together "some very significant projects" in the next six months. He added that conservation efforts could save up to five million barrels of oil by 1985.

In other respects, the plan is similar in balance to last year's version, placing heavy emphasis on nuclear energy, coal and production of synthetic oil and gas from coal and shale in the medium term.

As for nuclear power, the plan anticipates that installed capacity will grow from its present level of 39.6 GW to 70–76 GW by 1980, 160–185 GW by 1985, 265–340 GW by 1990 and 450–800 GW by 2000.

AUSTRALIA

Filling the information breach

From Sydney, Peter Pockley looks at Australia's science policy in light of the Science Department's recent Annual Report

SOMEWHAT dated and dented by more recent political and bureaucratic changes, the Annual Report of the Australian Department of Science and Consumer Affairs for the year July 1974 to June 1975 has now surfaced. The most visible dent on the Department is the recent removal of consumer affairs from its title and duties.

Nonetheless, this retrospective report is useful, if only for combining in one list the various activities which, under the now deposed Labor administration, had been brought under the one department. It is, however, as informative to note the omissions from this list as the inclusions, for Labor's Department of Science and Consumer Affairs exercised only partial coverage of the national science scene, a situation unchanged under the new regime. By international comparison, Australia spends in the government sector one of the highest proportions of total research funds (of the order of three-quarters). National figures are not yet available for the year in question, but a conservatively generous estimate puts the Department of Science's proportion of this expenditure (that is, funds directly controlled by the Department) at no higher than a quarter, probably less. At least three-quarters of "in-house" government-financed research was done outside the Department's control.

The most obvious organisation standing beyond the influence of the Department is Australia's colossus of research, CSIRO, with expenditure of \$143 million in 1974-75. In company with the newer and smaller statutory bodies, the Anglo-Australian Telescope and the Australian Institute of Marine Science, CSIRO remained responsible directly to the Minister for Science and Consumer Affairs, and not through his Department. (Again, this arrangement did not change with the change in government in December 1975.)

While a lack of mention of CSIRO in the Department's report is strictly correct in legislative terms (indeed, if the proudly independent CSIRO had been mentioned by the Department from which they keep a cool distance, there could have been a row of epic proportions), it means, unfortunately, that the report cannot be treated by local or international readers as representative of the national science scene.

It should also be added that sub-

stantial government-financed research and development efforts are also carried on outside the responsibility of the Science Minister. Notable among these are the Australian Atomic Energy Commission, the Bureau of Mineral Resources, and the Weapons and Aeronautical Research Laboratories, who all report to separate Ministers.

What the Science Department report does show, though, is the dominance within the Department, measured in terms of staff and expenditure, of five operational research and service units, each of which had been long established under other Departments before Labor placed them under the Department of Science from December 1972 onwards.

Out of a total of 3,368 established positions, these operational units had 3,097 and the central office 271. The Bureau of Meteorology (\$31.2 million), the Patent, Trade Marks and Design Office (\$5.7 million), the Antarctic Division (\$4.5 million), the Analytical Laboratories (\$2.7 million) and the small Ionospheric Prediction Service (\$600,000) accounted for 78% of the Department's overall expenditure of \$57.2 million. If \$8 million of payments to university researchers by the Australian Research Grants Committee are included, the proportion of these six activities rises to 92%. Through revenue earned, the Patent office was almost entirely self-supporting, and the Bureau of Meteorology earned over a third of its keep, the total revenue of the Department being \$17.5 million.

The Department also administers civilian space projects conducted through the operations of three tracking stations for the American NASA. Of the 400 employees in this work, only 40 are Australian public servants; the \$11 million cost in 1974-75 was met by NASA.

The Department can point to an apparently modest sum of \$2.6 million being spent on general administration. However, the operational units had largely self-contained administrations before their transfer to the Department, and the geographical separation from Canberra of most of them had probably not allowed much in the way of reduced administrative expenses. The Department talks of developing "staff resources without any significant increase in numbers", and of units becoming "more effective as a result of reorganisation". Personalities aside, it is not surprising that friction did develop between the central administration and some of its operational outposts; naturally none of this emerges in the report.

Policy influence

The constraints on an Australian Department Head in writing a report to his Minister are such that only the blandest information on matters of policy and planning is presented. For instance, Sir Hugh Ennor's report does note the formation of the Interim Australian Science and Technology Council (ASTEC) as "an important, but not exclusive, source of advice to the Government". The report adds that "the Department's work will both supplement and complement ASTEC;

AAT change

THE 3.9-metre Anglo-Australian Telescope (AAT) on Siding Spring Mountain, New South Wales, is to have a new Director in September. He is Professor Donald Morton, of Princeton University, and he succeeds Professor E. J. Wampler who left at the end of last month to return to the Lick Observatory in California. Wampler's appointment was for a period of two years ending in October, but by "mutual agreement" he has withdrawn early—mainly because he considered complete his task of seeing the AAT commissioned, but also, it is believed, because he was not completely happy about future prospects for the telescope at a time of economic stringency.

Not that the telescope is lacking funds, according to Professor Vincent Reddish, of the Royal Observatory Edinburgh, who sits on the AAT

Board. Its present annual budget is about £1 million, equally financed by the UK and Australia, and, says Reddish, all the indications are that it will continue to be well looked after by the two governments as an important ongoing project.

One of the most interesting aspects of the AAT is the way in which it has been operated hand-in-glove with the UK 48-inch Schmidt Telescope, located a few yards away on the same mountain. The Schmidt is essentially a survey instrument, and on most days AAT astronomers are to be seen in the Schmidt building poring over the most recent plates to find astronomical objects worthy of more detailed examination by the AAT. Much useful work on the spectroscopy of faint galaxies, and the dynamics of groups of them, for example, has been done by the AAT in tandem with the Schmidt.

Roger Woodham

in particular, it will act as an agent for ASTEC in obtaining and supplying data in conducting or commissioning studies and in carrying out those liaison and other tasks both within and outside Government that it is favourably placed to undertake". As far as the Department was concerned, the demarcation lines in the highly competitive business of influencing the government are kept nicely fuzzy. All this, of course, may be water under the bridge when the Liberal Government's current review of ASTEC is publicised and implemented.

In support of its own advisory role, the Department has pursued its data collecting activities. The long-running Project SCORE (Survey and Comparison of Research Expenditure) first surveyed expenditure and manpower in Australian research and development for the financial year 1968-69; the results were published in 1972 and 1973. Project SCORE is now reported to be "well underway" on "two-yearly intervals" and using OECD bases for statistics "to be sure that Australian figures may be compared with overseas figures".

The day after the report's period of survey concluded, that is, July 1, 1975, the 555-strong Patent Office was removed from the Department and transferred to the Attorney-General's Department (more recently still it has found another home in Business and Consumer Affairs).

It is easy for outside observers (and most insiders, too) to be bamboozled by the dispersed nature of Australia's scientific organisation. This is not, however, an argument for centralised control, but for at least one government sponsored effort to document in assimilable form the standing and overall progress of science on a national scale. The Science Department's report cannot claim to do this, nor to be fair does it claim to do so. Yet, not many scientists in Australia are more than dimly aware of what is going on in fields outside their own speciality. The interested public and politicians find the task equally tough, although they are helped by some very informative programmes on ABC radio.

The only organisation in Australia which has had the drive and capacity to fill this information breach is the interim ASTEC, but it is still on ice while the government reviews its aims and operations. If, however, ASTEC is allowed by Prime Minister Malcolm Fraser to survive in anything like its form under Labor which would make public reporting central to its functions, then the prospects of the scientific community and general public alike catching and keeping up with the hare of Australian research are quite bright. Or, is it a tortoise? □

USSR

Give and take

April, traditionally an auspicious month for Soviet scientists with the announcement of the Lenin Prizes, has this year brought an ominous picture for those out of favour with the establishment: two dissident scientists on trial, continuing harassment and repression of refuseniks, and Academician Andrei Sakharov briefly held with his wife in police custody. Vera Rich reports

THE recent "Omsk incident" involving Academician Sakharov marks a new development in the Soviet authorities' campaign against him—the loss of his personal immunity. It happened when Sakharov and his wife Yelena tried to attend the trial of Mustafa Dzhemilev, a young agronomist who has become the leader of the movement for the repatriation of the Crimean Tatars, deported *en masse* to Siberia in 1944.

"Political" trials in the Soviet Union are not normally open to the public, but Sakharov did sometimes manage to enter the courtroom as a friend of the accused—in the case of the mathematician, Dr R. I. Pimenov, in 1970, for example. By 1972, however, when Vladimir Bukovskii was on trial for having sent to the West detailed reports on Soviet misuse of psychiatry for political ends, Sakharov was no longer admitted, protesting instead outside the court. Now he and his wife have been prevented from doing even this.

According to Sakharov, who says two official TASS bulletins on the incident are false, plain-clothes KGB men prevented the friends and relatives of the accused from entering the courtroom, using "rude physical force", directed "in particular" at Sakharov and his wife. In an "immediate reaction" to the "mockery of the feelings of friends and relatives, the mockery of the law, the whole tragic circumstances of this case and other political cases in our country", Sakharov said he hit in the face "one or two KGB men" and a civil policeman who was acting on KGB orders. The Sakharovs were removed to police headquarters, where Sakharov made a statement claiming that the police were acting "on the side of the law-breakers". The next day, the Sakharovs tried once again to protest against the exclusion of the Dzhemilev family from the court, and again were taken to police headquarters.

Sakharov stresses that the civil police treated them "correctly", and says that earlier reports to the contrary were

due to a bad telephone connection to Omsk. He denies vehemently that he created a disturbance in the courtroom, as TASS had claimed. This was quite impossible, he says, since "three ranks of KGB men" prevented them even getting near the court. He admits that the possibility of criminal charges against himself is "not excluded".

Sakharov's attempt to focus world attention on the Dzhemilev trial in Omsk, a city closed to foreign journalists, came at the same time as his friend and close associate in the human rights movement, physicist Andrei Tverdokhlebov, faced similar charges of anti-Soviet activity in Moscow. Sakharov felt that the location of this trial in the capital, and the fact that Tverdokhlebov's membership of the illicit Moscow group of Amnesty International makes him a well-known figure, would ensure publicity abroad, whereas the Dzhemilev trial might pass unnoticed without his presence. Tverdokhlebov was sentenced to 5 years' exile; Dzhemilev received 2½ years in a strict regime labour camp.

As for the general position of dissident scientists, this shows little sign of improving. The Kiev seminar for Jewish refusenik scientists, one of the many off-shoots of the original Voronel seminar in Moscow, and led by physicist Vladimir Kislik, has been shut down. Mark Azbel, who now runs the Voronel seminar, has once again been refused a visa for Israel. And Professor Veniamin Fain, one of the very few activists and refuseniks to be allowed to continue in his academic career, has now been dismissed from his post at the Institute of Solid State Physics in Moscow.

Sakharov has meanwhile accepted an invitation to become a member of the executive committee of the Campaign Against Psychiatric Abuse (SAPA), the British section of the International Initiating Committee against Misuses of Psychiatry for Political Purposes. □

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IN BRIEF

Uranium meetings

The first ever talks between consumers, processors, and producers of uranium have been called to coincide with the annual general meeting of the UK Uranium Institute in London on June 15. The Institute, a "think tank" set up last year by uranium producers, admitted electrical utilities and nuclear fuel reproducers in January, and at the June meeting representatives are expected from Western Europe, Australia, Japan, North America and South Africa. They will discuss the physical and commercial factors influencing the uranium market and the issues they raise for the industry. In Vienna earlier this month, delegates from 38 states attending an International Symposium on the Exploration of Uranium Ore Deposits, arranged jointly by the International

Atomic Energy Agency (IAEA) and the Nuclear Energy Agency (NEA) of the OECD, heard that the discovery rate must increase from around 80,000 short tons a year to nearer half a million by the turn of the century if projected demands are to be met. The IAEA and NEA are now looking at the need for "a more permanent group of experts" to consider the best directions for research and development in exploration techniques.

Indian population measures

In an effort to combat the country's population growth, India's Minister of Health announced earlier this month an increase in the minimum marriage age, from 18 to 21 for men and from 15 to 18 for women. Compulsory national sterilisation will not be introduced for the moment, though indi-

vidual states will be allowed to enforce their own sterilisation measures for couples with three children. Incentives for voluntary sterilisation will include Government payments to couples of up to 150 rupees (around \$17); schemes introducing favourable priorities for jobs, accommodation and loans will be left to individual states. About 8% of federal government aid to states will be directed towards family planning; the amount states will receive will be based on 1971 population figures, as will the number of seats they hold in Parliament. Three states — Maharashtra, Haryana and Punjab—have already drafted legislation for compulsory sterilisation. India's population, estimated at 600 million, could top 1,000 million by the turn of the century if it continues rising at the present rate of 1 million a month.

APRIL 29, 1976 is the 150th anniversary of the historic meeting at which Sir Stamford Raffles proposed the formation of the Zoological Society of London, best known to the public because it established the London Zoo. Even then, however, the creation of a menagerie came second to "the advancement of Zoology and Animal Physiology" in the objectives of the society. For the last 150 years the society has continued its scientific work in addition to maintaining the collections of living animals in Regent's Park and, since 1931, at Whipsnade in Bedfordshire.

A visit to a zoo is generally looked upon as a harmless form of recreation, though there are those who voice moral objections to the display of captive animals. In the past, some zoos kept animals in unduly restricted cages, fed them on inadequate diets, and paid scant heed to their welfare. As a result disease was rife and survival short. In addition, populations of rare wild animals were often depleted or exterminated when collectors killed many more than they shipped back to maintain the collections. The Zoological Society of London was, from the first, aware of these dangers, and tried to improve the condition of their animals by studies of their physiology, nutrition and behaviour. Attempts were also made to breed as many as possible to reduce the pressure on the wild stock.

Even in 1877, when the Regent's Park zoo was being planned, efforts were made to house the animals properly: the architect employed by the society, Decimus Burton, divided his efforts between designing the Athenaeum Club for the literary and

scientific leaders of the period, and creating a house for the giraffes in the zoo. The club has changed little, but standards of animal accommoda-

Zoo celebration**KENNETH MELLANBY**

tion have improved considerably.

Zoos are used for systematic teaching of zoology as well as to show visitors the variety of the world's animal life. British children learn about the fauna of other lands, and many Africans see lions and other species seldom visible in their homelands, though recently the opening of zoos in Africa has changed this. Large crowds at the University zoo at Ibadan in Nigeria can admire a healthy pride of African lions, possibly unaware that they derive from a lion denoted by Longleat in Britain and a lioness from Dresden in East Germany.

Scientific meetings started even

before the zoo opened its gates. In April 1827 Dr Joseph Brookes lectured to a substantial audience on his dissection of an ostrich. The scientific journals of the society have continued to be published since 1830. Much of the earlier work was anatomical, using the corpses of animals which died, but there have always been studies of diet and behaviour aimed at improving the conditions of the exhibits. However, not every opportunity for research has been exploited: Sir Peter Chalmers Mitchell (best known for his efforts to establish Whipsnade) told me in 1936 how he had been approached by several ageing members of the aristocracy, who asked to be allowed to act as voluntary keepers of the larger mammals, in the belief that this would enhance their virility. It would have been interesting to have assessed the results; the society might even have gained a source of much-needed income!

Since the last war, scientific work has made further progress. The establishment of the Nuffield Institute of Comparative Medicine, the Wellcome Institute of Comparative Physiology and a modern Animal Hospital has provided unique facilities for research. These are well used, but more money will be needed if their potential is to be fully exploited. The society is now in a position to lead the world in work essential to the conservation of the many animals which are increasingly endangered by human population growth, industrialisation and pollution in their homelands. Conservationists and animal lovers need the Society today even more than they did 150 years ago.

correspondence

Genetics at the OU

SIR,—Since our veracity, objectivity and judgment have been called in question in a letter signed by Professor Pentz (April 8, page 479), which contests the criticism which we made of the genetics course now offered by the Open University, we hope you will allow us to put the record straight.

Much of the letter is polemical in the worst sense ("gratuitous insult", "prejudice", "mere opinion"). It also distorts our letter to mean what it does not say, namely, that we were attacking *all* Open University courses. In fact, we explicitly and specifically criticise the genetics course only.

The charges of falsehood made against us turn on the technicality of what constitutes "the Consultative Committee". The full Committee consisted of two members of the OU Course Team engaged in writing the course (Professors Rose and Jones), Dr C. Butler as Director of the Nuffield Foundation (subsequently replaced by the new Director, John Maddox), Professor H. Kornberg as a Trustee of the Foundation and five geneticists not involved in the writing of the course (Professors Jinks, Lewis, Pritchard, Sang and Dr Kacser). A sixth geneticist, Professor Bodmer, who was initially a member, later resigned.

It was these five geneticists who were actively engaged in meetings with the course team. In many sessions they made some far reaching criticisms and suggestions, including the proposal to reduce the coverage of the course radically (Consultative Committee meeting of October 20, 1975), but these were largely ignored. Professor Rose was the only member of the course team who attended all meetings. How much of our comments reached the rest we have no means of knowing. Eventually, and in despair, the five felt compelled to write a report to the Foundation who had appointed them.

This, quite properly, was confidential and could not have included any member of the course team. All other members received a copy. The debating point that they were not the full Committee, appears to have been introduced to conceal the reality of the situation. In a final attempt to improve the course, the Chairman of the Committee and the Director of the Foundation had a meeting with Professor Rose where the substance of our suggestions was discussed with him. The most important ones had, of course, been previously discussed in Committee. None of our major suggestions were accepted by Professor Rose.

The charge of "prejudice" turns out to be that the considered opinions of five geneticists with many years of teaching experience and great interest in teaching methods are set against the reports of some students that Units 1 and 2 were "OK", "very interesting" or "very easy". We are happy to leave this charge to the judgment of our colleagues.

We have gladly given of our time and experience for an enterprise which, at the outset, appeared to us both academically and socially important. In all our endeavours we had, and continue to have, only the welfare of potential students in mind. Because of this we must re-iterate that the students are ill served by the course as it stands. It is too long, too advanced and too detailed for second level part-time students and is inadequately prepared for non-OU Institutions. It would have been irresponsible to have brushed this opinion under the carpet even if our actions caused embarrassment to a member of the OU staff. Furthermore a venture which has absorbed a very large amount of money from a charity and, as we are now informed, an even larger amount of public money, should not be immune from informed criticism. There is nothing sacred about a Genetics Course—even an Open

University course—which is being actively promoted for sale to Universities at large.

Yours faithfully,

J. H. SANG

*School of Biological Sciences,
University of Sussex*

H. KACSER

*Department of Genetics,
University of Edinburgh*

R. H. PRITCHARD

*School of Biological Sciences,
University of Leicester, UK*

Science and the media in Canada

SIR,—In reading David Spurgeon's account of science and the mass media in Canada (February 5, page 353), I was interested in noting the abrupt injection of subjectivity in the otherwise quite objective summary, represented by the rather plaintive "how would they know?" comment, referring to popular scepticism of newspaper science articles.

I could cite many cases of science reporting in Canadian newspapers, and personal contacts with reporters, where the clear objective has been to overdramatise science stories, frequently through exaggeration, on the admitted grounds of necessity to catch the readers' eyes. Perhaps this is more acute in the case of environmental sciences (one reporter from a large daily wanted a concoction of disgusting material prepared to be photographed as an example of pollution in the Great Lakes).

Consequently, although I share Mr Spurgeon's dismay at the apparently poor job we are doing in informing Canadians of scientific matters, I must express some relief to learn of our sceptical public.

Yours faithfully,

ROBERT K. LANE

*Edmonton,
Alberta, Canada*



Competition 7

A PRESENT day headline for Charles Darwin's achievement might read "British scientist finds life's secret on tropical island".

A prize of £10 is offered for the best modern headline for any scien-

tific achievement past or present. Inflated claims or deceptive packaging are welcome. The closing date for entries is June 8.

Competition 6 required a rhyming couplet to help familiarise an SI unit. M. Hammerton of Newcastle receives honourable mention for a McGonagall verse, as does P. A. Mohr of Cambridge, Mass., for his brave

efforts to keep the Imperial system alive:

The 3,000 metres steeplechase
Is a two miles less one-and-a-twelfth
furlongs race.

But the clear winner was John Gribbin of Brighton with:

A Pascal of pressure on top of your
head

Is the same push that butter exerts
on sliced bread.

news and views

Does the key fit the locks of the past?

from Peter J. Smith

FEW would now disagree with the view that the plate tectonics hypothesis has provided a most successful model of the tectonic activity now taking place at the Earth's surface. Obviously there are still many unresolved problems, not the least of which, as Helwig has demonstrated recently (*Nature*, **260**, 768; 1976), is the clarification of the processes through which the collision of plates is translated into orogeny. Nevertheless, the basic outline is there, and for the time being there is little reason to suppose that the filling in of the detail is more than a matter of practical application, however difficult that might turn out to be. As Le Pichon *et al.* (*Plate Tectonics*, Elsevier, 1973) have pointed out, the supreme advantage of the plate tectonics hypothesis is that "it is predictive and can be tested by measuring relevant parameters."

But the real problems start with the attempt to extrapolate conventional plate tectonics backwards in time. Some of the difficulties are fairly obvious. An important part of the evidence for modern plate activity comes, for example, from the horizontal and vertical distribution of earthquake hypocentres. But there is no way of directly observing past earthquake distributions; ergo, important evidence for past plate activity is unobtainable. Other types of evidence, such as that from oceanic magnetic lineations, are in principle more permanent but in practice of very little help. As everyone now knows, ocean floors have an unfortunate tendency to disappear down subduction zones, destroying most of their history with them. As most constructive ridges and transform faults occur in oceanic crust, the chances of examples of either plate boundary having survived the past 200 million years are remote.

The burden of evidence in favour of the past existence of active plates must therefore rest on the identification of examples of just one of the three types of plate boundary—the consuming margins which have some direct association with continents. But in addition to the practical problems, this raises what some would no doubt call a philo-

sophical issue. The successful identification of old subduction zones is (or, in deference to the cynics, should be) rather dependent upon there having actually been subduction zones in the past. Cady (in *Implications of Continental Drift to the Earth Sciences*, Academic Press, 1973) has remarked that not only do "the earmarks of subduction become blurred with time" but they "may be recognised only by those who believe the present to be an invariable key to the past". Thus Le Pichon and his colleagues, whilst appreciating the difficulty in testing models of older plate tectonic processes and being sceptical of attempts to do so, nevertheless claim that "there is no reason to suspect that there has been any change in the major causes of deformation of the earth during the past billion years or so". This is a view commonly expressed and appears to be based on an ingrained reluctance among Earth scientists to question the tenets of uniformitarianism, however vaguely understood. But is it a valid statement? Is there really no cause to suspect?

There is probably little cause for suspicion about the past 200 million years. The difficulties of identifying Mesozoic fossil plate boundaries are far from negligible, but it is at least evident that throughout this period the present continents were drifting, ocean floors were spreading and plates were interacting much as they are now. The most convincing example of a Mesozoic subduction zone is perhaps the Great Valley region of the western United States. To the west of the Valley lies the Franciscan Formation, a chaotic mélange apparently deposited on oceanic crust. But the Great Valley sequence itself, separated from the Franciscan by a long sharp thrust fault system but spanning the same time interval, is a more orderly succession of miogeosynclinal rocks overriding part of the mélange to the west and resting on continental crust in the east. As Hamilton (*Geol. Soc. Amer. Bull.*, **80**, 2409; 1969). Ernst (*J. geophys. Res.*, **75**, 886; 1970) and others have argued, this is a trench-like environment probably representing an ancient

site of Pacific plate subduction; and Dickinson (*Rev. Geophys.*, *Space Phys.*, **8**, 813; 1970) has even reconstructed the position of the sloping palaeoseismic zone on the basis of potash values in the Sierra Nevada which stretches to the east.

But in respect of the Palaeozoic and Precambrian, matters may not be even this simple. Piper (*Earth planet. Sci. Lett.*, **28**, 470; 1976 and elsewhere) has shown palaeomagnetically that Africa and North America apparently acted as a single unit from 2,700 million years ago to the breakup of Pangaea—a conclusion strongly implying, but far from proving, that Pangaea itself existed for at least that period. Morris (private letter), whilst not necessarily disagreeing with Piper's conclusion, believes that both the quality and quantity of palaeomagnetic data are insufficient to prove the case; and McGlynn *et al.* (*Nature*, **255**, 318; 1975) have produced data which seem to refute it. The problems in obtaining reliable palaeomagnetic directions, accurate ages and thus polar wandering paths from Precambrian rocks are well known. Nevertheless, the balance still seems to be in favour of a single supercontinent for at least the 2 billion years before the onset of Wegenerian drift.

If this is confirmed it might have some significant implications. The environment represented by a single continent, whether or not surrounded by a spreading ocean floor, is rather different from that represented by a number of continents interspersed by spreading ocean floors. It is by no means certain therefore that plate-tectonic orogenic hypotheses derived from experience of the latter environment (which involves the interaction of rigid plates whose rigidity can be observed) would be directly applicable to the former. Equally, of course, it is by no means certain that such an extrapolation would not be possible. The point is that there is some reason to suspect that there could have been a change in the major causes of deformation with time, even though such a view could be held in some sense to weaken the strict uniformitarian doctrine. In practice, so strong has been

the urge to accept uniformitarianism that there has often been a tendency not to debate the rights and wrongs of applying conventional plate-tectonic orogenic models to an alien environment but to change the environment to fit the hypothesis. Hence the invention of pre-Mesozoic intercontinental oceans—for example, the proto-Atlantic Ocean to explain the Palaeozoic Appalachian-Caledonian orogenic belt in terms of continent-continent collision. Presumably this sort of circular argument is partly why Le Pichon *et al.*, despite their previous statement, regard the attempt to explain older geological features in terms of plate tectonics as having “a certain dream-like quality”.

The only person I know to have questioned uniformitarianism explicitly is Sutton (see, for example, Sutton, *Geol. Teaching*, 1(1), 14; 1976, and Sutton and Watson, *Nature*, 247, 433; 1974) who has modified the sacred doctrine with an element of evolution, chiefly the idea of a gradual increase in crustal rigidity with time. In Sutton's model the present phase of rigid plate activity, which began to develop about 1 billion years ago, was preceded by about 1.5 billion years of activity involving small permanent plates each a few hundred kilometres across. These small plates were aggregated into “a few large continental sheets which were themselves moving as essentially coherent units” and were separated within the sheets by mobile belts. Such a scheme obviously gives ample scope

for the existence of intercontinental oceans; but if it were to be modified in terms of a single supercontinent, the interesting question would arise as to the extent to which the internal mobile belts would be flexible enough to allow internal spreading ocean floors to develop. For upon this might depend the validity of extrapolating current orogenic hypotheses to the pre-Mesozoic mountains which undoubtedly exist.

There is evidence both for and against pre-Mesozoic internal oceans. Taking all the ‘favourable’ evidence together, however, it may be in order to wonder whether the support it is said to give to ancient internal oceans may not also be based on circular reasoning. Broadly, the existence of ancient subduction zones is inferred from the recognition of rock types (such as mélanges and blueschists) characteristic of modern subduction zones. But there is no reason in principle why different tectonic environments might not give rise to some similar rock types in different, though perhaps closely related, ways. Orogenic belts in two different environments may be due to causes similar enough to involve the formation of similar rocks, but not necessarily so alike that they need both involve subduction zones and plate collisions. To argue otherwise would surely be to adopt a form of uniformitarianism so strict that it would probably deny the possibility of two different tectonic environments in the first place. □

coding for 28S ribosomal RNA in *Drosophila melanogaster* is sometimes divided into two subunits separated by a non-transcribed spacer.

The most advanced dissection of repetitive gene sequences is that of Brown on the *X. laevis* genes for 5S rRNA and 40S rRNA. In each case they are arranged as tandem repeats of a gene sequence and a non-transcribed spacer sequence. The transcribed sequences are all identical whereas the spacers show some variation in length. Their individual lengths are determined by the number of component small subunit sequences included in each spacer. In somatic cell rDNA this heterogeneity in length has been localised to two distinct regions of the spacer sequence.

How is this spacer length variation distributed throughout the genome? The cloning of a tetramer fragment of a 5S rRNA repetitive gene has given Brown sufficient starting material to determine whether adjacent spacers have similar lengths. They did not. It is therefore most unlikely that the evolution of the tandem genes involved anything like a rolling circle mechanism.

The soundness of that conclusion and of any others drawn from the use of cloned fragments will depend in part on the fidelity with which the initial fragment is copied during cloning. H. M. Goodman (University of California, San Francisco) provided experimental assurance that assumptions of fidelity are, in fact, valid. He has cloned mouse mitochondrial DNA using a pSC101 plasmid vector in an *Escherichia coli* host and has compared the original DNA with that recovered after 25 generations of the host. On the basis of various criteria including restriction enzyme fragmentation patterns, electron microscopic contour length determinations and melting curve analysis, the cloned DNA was identical to its ancestor within the limit of detection of one base pair mismatch in 300.

There were two reports in addition to that of H. M. Goodman of the cloning of mammalian genes. One was a very brief mention by K. Murray (University of Edinburgh) of the cloning of mouse satellite DNA using a phage lambda vector; the other a full presentation by T. Rabbitts (MRC Laboratory of Molecular Biology, Cambridge) of his success in cloning a partial DNA copy of purified rabbit globin mRNA, using a mini-ColEI plasmid vector (see *Nature*, 260, 221; 1976).

Gene cloning is clearly an established technique. The same cannot be said for the expression of cloned genes, an aim close to the heart of commercial genetic engineers who hope eventually to

Genetic engineering

by Peter Newmark

A meeting on Genetic Engineering was held at the University of Glasgow on March 22–23, 1976. It was the 2nd Tenovus (Scotland) symposium of the Nucleotide Group of the Chemical and Biochemical Societies.

“GENETIC Engineering” is a fine title by which to attract the maximum attendance to a symposium. It is for some a sinister, for many an exciting prospect; it not only is seen by many molecular biologists as the path to their second coming but is also attracting considerable commercial interest.

Those who made the trip to Glasgow hoping for evidence of their wilder hopes or fears must have come away disappointed, for the meeting illustrated better the potential than the product of the techniques of cloning recombinant DNA.

The only real applications of cloning to be presented were in rela-

tion to the study of repetitive gene sequences. That topic was covered by D. D. Brown (Carnegie Institute, Baltimore), S. G. Clarkson (Zurich) and D. Glover (Imperial College, London). They have all been investigating the organisation of the hundreds or thousands of identical copies that exist for certain genes in eukaryotic chromosomes. The study of restriction enzyme fragments of repetitive genes has already made clear the basic organisation of their tandem repeats.

A finer analysis, however, of these naturally “cloned” genes has resulted from the experimental cloning of their restriction enzyme fragments. The availability of cloned fragments facilitates the preparation of heteroduplex DNAs for hybridisation and electron microscopic analysis by gene mappers. Clarkson has been able to place the *Xenopus laevis* gene for methionyl tRNA_i within a particular 1,900 base pair sequence. And Glover has discovered that the DNA sequence

be able to harvest gene products such as interferon. But in "Will bacteria produce useful proteins from inserted eukaryotic genes?", N. H. Carey (Searle Research) was far from being able to answer his own question and J. Atkins (ICI) was not very much nearer. The latter is working with a phage lambda vector and a K. Murray host, a combination that at least has resulted in the successful translation of cloned prokaryotic genes, for example the production of penicillinase from the *Bacillus licheniformis* gene cloned in *E. coli*.

Another example of successful functioning, this time without translation of a cloned gene came from H. M. Goodman. In a futuristic vein he presented evidence that it was possible to insert into the PM9 plasmid a chemically synthesised, double-stranded, 21 base pair DNA sequence of the *lac* operator. Transfection of *E. coli* with that recombinant DNA resulted in the constitutive production of β -galactosidase by some cells. The conclusion is that the plasmid, replicated to the extent of about 30 copies per cell, provided enough cloned copies of the *lac* operator to bind the cell's six repressor molecules.

Many of the speakers paid tribute to the technological contributions of E. Southern (University of Edinburgh) to their own work. E. Southern himself described both his "gene machine"—a high capacity, preparative agarose gel apparatus allowing 30-fold purification of restriction enzyme fragments—and his transfer technique. The latter technique involves the transfer of DNA fragments, banded in agarose gels, to cellulose nitrate filters on which they can be identified by hybridisation techniques.

Tribute should also be paid to N. M. Wilkie (University of Glasgow) who

told of his mapping of the herpes virus genome. By admitting his desire to clone herpes fragments he provoked a discussion on the hazards of cloning that had threatened never to surface. No amount of argument, however, could answer D. D. Brown's question of whether the cloned fragments of the herpes gene were any more hazardous than the infectious virus itself, and if not why prohibit the cloning? That particular problem is one of assessing the unpredictable and improbable hazards of cloned fragments against a known enemy. For the moment the fear of the unknown prevails. □

Fossilised fury

from Barry Cox

ANALYSES of the mechanical adaptations of skeletons for movement and support, or of teeth for different kinds of diet, are comparatively simple. The interpretation of other structures, once used in the intraspecific social interactions of extinct animals, is far more difficult. Even in modern mammals, such a basic analysis as that of Geist on the evolution and use of horns is still only 10 years old. Where such living analogues are available, vertebrate palaeontologists are using these to explain some structures found in extinct animals, and are using similar techniques to explain other, more bizarre structures which find no obvious parallels in the world today.

In fact, Davitashvili's book *The Theory of Sexual Selection* (1961) included many suggestions as to the possible functions of unusual structures in fossil reptiles, but few non-Russian workers have read it. Some of his ideas have been independently put forward by other workers: for example, Galton (*J. Paleont.*, **45**, 40; 1971) has suggested that the greatly thickened skull roof of the pachycephalosaurian dinosaurs was correlated with the use of the head in pushing and ramming during intraspecific combat, like that seen today in mountain sheep.

Geist himself suggested a similar function for the thickened heads of the herbivorous dinocephalian mammal-like reptiles, and Barghusen (*Paleobiology*, **1**, 295; 1975) has now convincingly shown that many other features of their skulls support this interpretation. Strong arches of bone transmit the impact force from the dorsal surface of the head to the occipital condyle. The line of action of this force also passes very close to this condyle, so reducing the torque that such blows would have produced at that joint. Barghusen also extends this explanation

to the carnivorous dinocephalians, suggesting that, as in living suids, head butting and shoving allowed combat while reducing the danger of mutual injury from the massive incisor and canine teeth.

Geist (1966) clearly demonstrated that the primary use of the horns of many living herbivores is for intraspecific mating combat or the defence of territory, rather than for defence against predators. Farlow and Dodson (*Evolution*, **29**, 353; 1975) have now used this approach to analyse the horns and frills of the ceratopsian dinosaurs. They suggest that the most primitive ceratopsians, such as *Protoceratops*, used their small nasal horn for lateral blows against the flanks of their opponents, and used the bony frill that extended upwards and backwards from the skull as a visual dominance rank display structure, like the antlers of deer. Some later ceratopsians had a long frill and paired, diverging horns. Farlow and Dodson suggest that the frill would have been prominently displayed when these animals lowered their heads, and that the horns of rivals would have interlocked, leading to a trial of strength—again as in deer. Other ceratopsians, which had unpaired nasal horns and a short frill, they compare with rhinos, in which there is less display, the horns do not interlock, and injury is more frequent.

The strange cranial specialisations of the hadrosaurian dinosaurs have long been a fascinating problem, for they are unlike any structures found in living animals. In some, the bones of the nasal region form solid crests or humps, or are excavated around the nostrils; in others, these bones form elongated crests enclosing the nasal passages. Davitashvili originally suggested that the solid crests or lumps were combat structures and that the hollow elongate crests functioned as species recognition signals in courtship both visually and, by acting as resonating chambers, also audibly (as earlier suggested by Wiman).

Hopson (*Paleobiology*, **1**, 21; 1975) has now examined the implications of these ideas. He points out that if they are correct, various predictions should be fulfilled. For instance, as would be expected, the shape of the crest, being the result of independent selection for behavioural functions, is not merely a reflection of the size and shape of the internal cavity. The crests also appear to be dimorphic; otherwise identical skulls show variation in the size of the crest, and presumably belong to different sexes, while different sexes bear characteristically different crests. These species recognition features also, as would be predicted, are better developed in those faunas which contain a greater number of species of hadrosaur.



A hundred years ago

MR. TOMLINSON'S remarks on safety-matches in *NATURE*, vol. xiii, p. 469, reminded me that, not long ago I accidentally kindled one of those matches by rubbing it on the edge of a Wedgwood-ware mortar. This material appears even better adapted than those mentioned by Mr. Tomlinson for igniting such matches, and I found that a common earthenware dish (glazed inside) answered the same purpose admirably. I tried to ascertain the degree of certainty with which a safety-match could be kindled by friction against these two materials, and was surprised to find that they are little inferior in this respect to amorphous phosphorus itself.

from *Nature*, **13**, 512; April 27, 1876

According to Hopson, the excavations around the nostrils of some hadrosaurs were filled with soft tissue, which could be inflated as a display structure and might also act as a resonator (as in elephant seals). He believes that the function of the elongate, hollow nasal tubes of other hadrosaurs was to provide a permanently dilated resonating system while at the same time providing a visual threat display.

The work of Geist and Davitashvili has provided a useful new approach for vertebrate palaeontologists. Though they cannot employ the experimental or observational methods in testing such hypotheses on fossil material, such techniques as Barghusen's analyses of associated structural adaptations, or Hopson's prediction-testing method, can provide adequate alternatives, and it is to be hoped that yet more fossil humps and bumps will find a function in the future. □

Liposomes to lysosomes

from a Correspondent

Now that many of the enzyme deficiencies which cause the lysosomal storage diseases are known, efforts are increasingly being directed towards finding methods of therapy. One obvious approach is to replace the defective or absent enzyme (see for example, *Enzyme therapy in lysosomal storage diseases*, edit by Tager *et al.*, North Holland/American Elsevier, 1974). These diseases seem to be particularly suitable candidates for enzyme replacement therapy because extracellular macromolecules and particles are taken up by the process of endocytosis (phagocytosis) into the lysosomal system. Thus enzymes supplied (to the blood stream) may be expected automatically to reach the intracellular location where they are needed. They still, however, have to be directed to the particular tissue or tissues where most lysosomal storage occurs and these (brain and muscle, for example) often do not carry out much endocytosis. In order to remove the substances that have already accumulated and to maintain the corrected condition a regular supply of enzyme is required. Immunological complications arise from repeated injection of foreign antigens (either the enzyme itself or other substances copurified with it).

The idea of injecting enzyme entrapped in microvesicles, gel particles or red cell ghosts has recently been investigated. A non-antigenic vesicle would not only prevent the enzyme from eliciting an immunological res-

ponse but would protect it from degradation before it reaches the required site. Liposomes, which are synthetic vesicles made of concentric bilayers of lipids alternating with aqueous compartments in which the enzyme can be carried seem to be useful vehicles because they are non-antigenic and biodegradable and their dimensions and constituent lipids can be adjusted to suit requirements. Attempts are now being made to direct liposomes to target organs by coating them with substances which are known to have an affinity for and (or) be taken up by a particular tissue.

In a recent paper Cohen *et al.* (*Biochemistry*, **15**, 452; 1976) describe *in vitro* experiments on the entrapment of N-acetyl hexosaminidase A (the enzyme deficient in Tay-Sachs disease) by liposomes and the uptake of these liposomes by the polymorphonuclear leukocytes of a patient with Tay-Sachs disease. Selective endocytosis was encouraged by coating the liposomes with aggregated immunoglobulin (IgG), a successful idea based on the known uptake of immune complexes by polymorphonuclear leukocytes (Weissman *et al.*, *J. exp. Med.*, **134**, 149s; 1971). The liposomes were shown to be taken up by the lysosomes and N-acetyl hexosaminidase A was detected in the 'corrected' cells.

This type of *in vitro* experiment merits further study, not only to investigate the fate of the entrapped enzyme and the liposomal material, but also the breakdown of the accumulated substrate. The question of access of the liposome-entrapped enzyme to the 'residual bodies' of the lysosomal system where much of the stored material is found, needs to be resolved. Furthermore, as it is known that activity of N-acetyl hexosaminidase towards the natural substrate, the ganglioside GM₂ is lost on purification (Srivastava *et al.*, *J. biol. Chem.*, **249**, 2043; 1974) it will be of great interest to see whether activity towards the natural substrate can be restored by replacing the purified enzyme within the cell.

While few animal models are available for the study of these diseases, *in vitro* experiments of this kind are clearly useful model systems. At the same time an investigation into the safety of the injected ingredients is of paramount importance and this can be done most conveniently using experimental animals. The release of lysosomal acid hydrolases which seems to occur parallel with the endocytotic uptake is a side effect that should be considered further. It is of interest to note that some of the pharmacological effects of liposomes are currently being investigated (Bruni *et al.*, *Nature*, **260**, 333; 1976).

Successful treatment of Tay-Sachs disease as well as other infantile diseases with neurological involvement seems, however, unlikely to be practicable. The difficulty of penetrating the blood-brain barrier, a prerequisite for removing accumulated substrate from the brain is problem enough. Also substantial accumulation and possibly irreversible damage have already occurred as early as 16 weeks gestation (Schneck *et al.*, *Pediatrics*, **49**, 342; 1972). Treatment of juvenile and adult onset diseases (such as Fabry's disease) seems on the other hand to be a more hopeful and justifiable proposition. □

Triton interactions

from P. E. Hodgson

THERE have been rather few studies of the interaction of tritons with nuclei, mainly because tritons are highly radioactive, with a half-life of about 12 years. Very stringent safety precautions have to be enforced, so work with tritons is usually done in Government laboratories. A series of measurements of the differential cross sections for the elastic scattering of tritons by nuclei was made some years ago at Aldermaston, and analysed to give triton optical model potentials. There were hardly any measurements of triton polarisation and these were of low accuracy, so very little was known about the spin-orbit term in the triton optical potential.

Recently a polarised-triton source has been installed on the Los Alamos Scientific Laboratory Tandem Van de Graaff accelerator, and this has made possible new measurements of triton elastic scattering of a higher accuracy than ever before. The Los Alamos group have measured the differential cross sections and polarisations for tritons elastically scattered from several nuclei, and analysed the results with the optical model (*Phys. Rev. Lett.*, **35**, 1623; 1975).

It is possible to calculate the triton optical potential by averaging the known nucleon-nucleus interaction over the three nucleons comprising the triton. This folding model, as it is called, has already been used successfully to calculate the optical potentials for deuterons, helions, and alpha particles, so it is important to test it for tritons as well.

The folding model gives for tritons an optical potential that is about three times as deep as the nucleon optical potential, has about the same radius and is slightly more diffuse in the surface region. The two neutrons in the triton are anti-aligned, so that the spin-

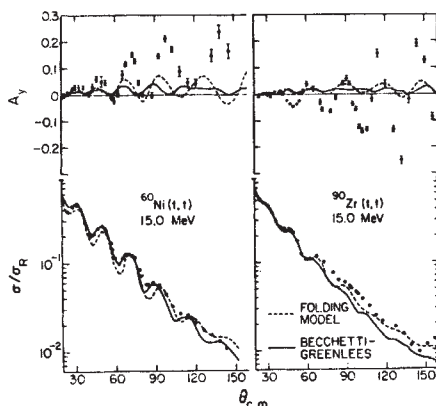
orbit force between a triton and a nucleus is the same as that between a nucleon and a nucleus.

Now the spin-orbit force is written as $V_{so}(r)\mathbf{L}\sigma$, where σ is the spin of the projectile and $\mathbf{L}$ the relative angular momentum between the projectile and the target. For the same incident velocity this angular momentum is three times greater for a triton than for a nucleon, so we expect the spin-orbit potential depth to be one-third the nucleon value to leave the whole potential the same. Since the depth of the nucleon spin-orbit potential for nucleons is about 6 MeV we expect a value of about 2 MeV for the triton spin-orbit potential.

To test this prediction, the differential cross sections were first compared with calculations using a triton optical potential obtained by folding, and the parameters were slightly altered to optimise the fit. As shown in the figure, this gives a good fit to the cross section. The polarisations calculated with a spin-orbit potential of 2.5 MeV are however far smaller than the measured values. Further calculations were made using optimum potentials obtained by Becchetti and Greenlees, with very similar results.

The parameters were then varied to try to fit the polarisations as well as the differential cross sections, and it was found necessary to alter the absorbing part of the potential as well as the spin-orbit potential itself. Potentials were found that fit the polarisations very well, but they have spin-orbit depths of 6 MeV, which is much greater than that given by the folding models.

The new data can therefore be



Polarisations (top) and differential cross-sections (bottom) for the elastic scattering of 15 MeV tritons by ^{60}Ni and ^{90}Zr compared with optical model calculations with a folding model potential (dashed lines) and with a Becchetti-Greenlees potential (full lines). Both potentials fit the differential cross-sections quite well, but give polarisations that are far too small.

successfully analysed by the optical model, but the strength of the spin-orbit potential is unexpectedly high, and poses a challenging theoretical problem. $\square$

Disturbed habitats

from Peter D. Moore

ONE of the basic aims of ecological research is the generation of theoretical models on the basis of which predictions can be made. The complexity of interactions in any ecological system make this a difficult task, but it is a particularly necessary one especially where a basis is required for the formulation of an informed management programme for a given habitat. As a consequence of this need, there has been a noticeable swing in ecological research in recent years away from the traditional study of 'natural' ecosystems to an unashamed interest in those habitats which have been subjected to some disturbance or perturbation at the hands, or feet, of man.

A good example is that of sand dune habitats, where the bulk of ecological research interest is no longer focused on the general course of successional process, but has become concentrated on the effects of trampling upon soils and vegetation. Some recent work in this field by Liddle and Grieg-Smith (*J. appl. Ecol.*, **12**, 893 and 909; 1975) has involved both descriptive and experimental studies on the Abeffraw sand dunes in Anglesey.

Examination of soils revealed that several changes occur with trampling. Trampled soils were found to have increased in their bulk density and, as might be predicted, the effect of a car was considerably more severe than the passage of a human being, the density being increased by a factor of about 30% more after a car has passed than after a man. Soil compaction was associated with higher water content, which could favour certain species sensitive to water stress in this arid environment, but the anaerobic nature of such soils would not encourage extensive root growth by most species.

Examination of the vegetation from transects across paths showed that although species number decreased towards the path centre, diversity remained fairly constant. This suggests that loss in richness is accompanied by an increase in evenness of apportionment of biomass between species; this was probably caused by reduction in dominance with increased trampling pressure. Other observations show a fall in total biomass and an increase in

the proportion of that biomass belonging to monocotyledonous species as trampling increases.

Unfortunately the vegetation data suffer from the disadvantage of having been gathered in June, when most of the winter annual species (mainly dicotyledons) would have already flowered and died. Since many of these species flourish under disturbed conditions, especially if sand is laid bare, they are likely to be an important winter and spring component of the path flora. Liddle and Grieg-Smith suggest that monocot:dicot ratio could prove a simple and useful index of trampling intensity, but seasonal factors need to be considered since there will be a greater proportion of dicots in winter.

Liddle (*Biol. Conserv.*, **8**, 251; 1975) has attempted to generalise yet further concerning the vulnerability of vegetation to trampling, on the basis of his sand dune data and further information derived from other habitats. He demonstrates a linear relationship between the logarithm of the number of people needed to pass along a linear path through a given vegetation type in order to reduce its biomass or cover by half, and the logarithm of the recorded primary productivity of that vegetation type. This relationship is not surprising, since more productive vegetation may be expected to regrow after trampling and so recover its former biomass more quickly. Liddle also considers that mature ecosystems are more resistant to trampling than early successional ones because they are more productive. This is not consistent with his own data, however, which show that short turf grassland containing such species as *Festuca rubra*, *Bellis perennis*, and *Poa pratensis* are particularly resistant to trampling yet are not mature in the successional sense, unless one regards the path community as a 'trampling climax', in which case the argument becomes a semantic one. The productivity/trampling resistance relationship may be genuine, but there is a danger of confusing net and gross ecosystem production when making successional inferences. Net ecosystem production does not rise during the early stages of succession, but then falls to zero at climax. Some of the most productive plant species are in fact found in immature ecosystems. It is therefore unwise to state that mature ecosystems are more productive, and therefore, more resistant to trampling.

The development of predictive models, or even the derivation of simple indices for the monitoring of man-induced changes is still some way off. But if Liddle's ideas on productivity are sufficiently compacted to hold water then we should be able to increase an ecosystem's tolerance to trampling simply by fertilising it. $\square$

articles

Variability of AO 0235+164 at radio, optical, and infrared wavelengths

The most recent outburst of the BL Lacertae object AO 0235+164, in November 1975, was particularly large and built up very quickly. These three papers present measurements made at radio, optical and infrared wavelengths, and discuss the extent to which the emitting regions in each case are associated.

Variability of AO 0235 + 164 at 2.8 cm.

THE radio source AO 0235+164 was discovered by Hazard and Sutton during lunar occultation observations at the Arecibo Observatory¹. Spinrad and Smith² classified the optical object as of the BL Lacertae type. It is a moderately bright variable stellar object with an off-centre nebular extension to the south. In this issue of *Nature* Rieke *et al.*³ report extensive optical observations which demonstrate the extraordinary nature of this object. The recent behaviour of the source at radio wavelengths is described here and in an accompanying paper by Ledden *et al.*⁴.

AO 0235+164 was added to the list of sources observed regularly in the variable source programme at the Algonquin Radio Observatory⁵ (ARO) in October 1972. Since then its flux density has been measured about once a month. The results are given in Fig. 1 and Table 1. The methods and errors of observation have been discussed by Medd *et al.*⁵.

There have been at least three major outbursts in three years, the last of which, peaking in November 1975, had an extremely large and rapid increase. The ratio of maximum (7.6 Jy) to minimum (0.6 Jy) flux density observed for this source is ~ 12 . This ratio is by far the largest we have observed

Table 1 Flux densities (Jy) of AO 0235+164 at 2.8 cm

Date	$S_{2.8}$	Date	$S_{2.8}$
1972		October 17	2.13
November 18	1.99	November 28	2.49
December 6	1.97	December 13	2.78
1973		1975	
March 22	2.89	February 6	3.38
April 19	3.73	March 16	2.41
May 8	3.23	April 12	2.00
June 22	2.62	May 27	1.53
July 22	2.13	July 30	1.74
August 24	1.84	July 31	1.71
August 27	1.90	September 3	3.58
September 14	1.96	September 4	3.51
September 15	1.97	November 19	7.43
October 31	1.47	November 25	7.67
December 11	1.09	December 7	6.47
December 13	1.07	December 16	5.92
			5.80
1974			5.75
January 18	0.85		5.88
March 4	0.74	December 17	5.70
April 12	0.60	December 19	5.58
May 23	0.64		5.48
June 25	0.95	December 20	5.59
July 4	0.98		5.54
August 22	1.66	December 21	5.48
September 2	1.75	December 22	5.36
October 15	2.20		

for any source at 2.8 cm, the next largest being ~ 6 for BL Lac.

Such behaviour seems typical of BL Lac objects. The majority of other BL Lac objects in the ARO programme also show rapid variations (time scales $\lesssim 1$ yr) and $S_{\max}/S_{\min}$ ratios in excess of 2 (for example, PKS0048-09, DA55, OJ287, OT081, BL Lac) though a few do not (PKS0735+17, PKS1514-24) (B. H. Andrew, G. A. Harvey and W. J. Medd, in preparation).

Measurements of the flux density of AO 0235+164 during the period December 16-23, 1975 (Fig. 2) showed no evidence of daily variations > 0.1 Jy, though variations of 1 or 2 Jy have already been detected on this time scale in both OJ 287 (ref. 6) and BL Lac (ref. 7) on several occasions.

Fig. 1 The flux density of AO 0235+164 at 2.8 cm since November 1972.

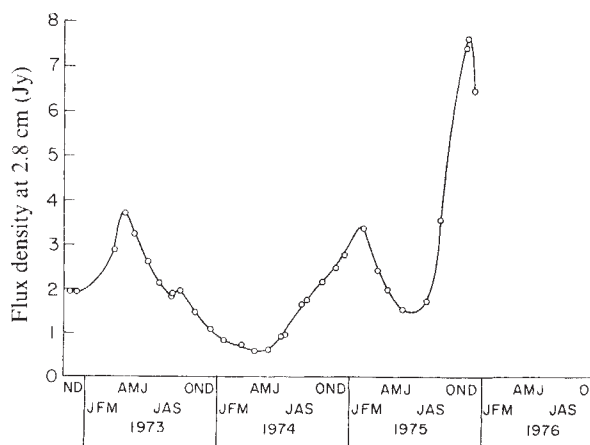
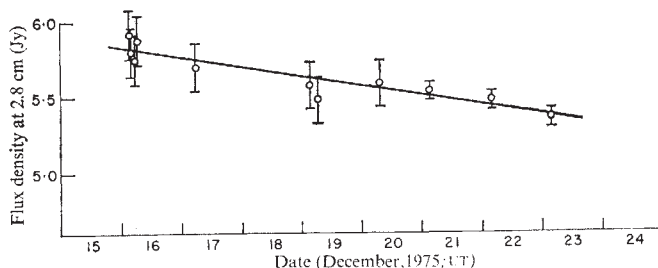


Fig. 2 The flux density of AO 0235+164 at 2.8 cm from December 16-23, 1975. There is no evidence for rapid variations superimposed on the gradual decay of the event of November 1975.



The Algonquin Radio Observatory is operated by the NRC of Canada. We thank Dr A. E. Neill for drawing this source to our attention, Drs P. C. Gregory and W. H. McCutcheon for making several of the observations in December 1975, and Dr P. A. Feldman for an observation in November 1975.

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⁴ Ledden, J. E., Aller, H. D., and Dent, W. A., *Nature*, **260**, 260-252-754 (1976).

⁵ Medd, W. J., Andrew, B. H., Harvey, G. A., and Locke, J. L., *Mem. R. ast. Soc.*, **77**, 109 (1972).

⁶ Kinman, T. D. *et al.*, *Astro. J.*, **79**, 349 (1974).

⁷ Andrew, B. H. *et al.*, *Astrophys. J.*, **191**, 51 (1974).

Radio spectrum of the major outburst in the BL Lacertae object AO 0235+164

THIS paper presents an analysis of the evolution of the radio emission of AO 0235+164 during the recent large outburst which coincided with the optical and infrared event reported by Kinman and Rieke¹. The event seems to be the clearest case to date of a correlated radio and optical outburst. Spinrad and Smith² suggested that AO 0235+164 is a red BL Lacertae object and noted that it is a radio and optical variable. Two smaller radio outbursts have been observed since 1972.8 by MacLeod *et al.*³ and, based on their data and that of J. C. Webber (private communication), the quiescent flux density level of the source at frequencies > 1 GHz is < 1 Jy (1×10^{-26} W m⁻² Hz⁻¹). The recent event described here offers an unusual opportunity to study an isolated outburst because its amplitude was much larger than the quiescent flux level, good coverage in time at two radio frequencies was obtained, and simultaneous observations over a wide range of radio, infrared, and optical frequencies were made.

The radio flux density data are presented in Tables 1 and 2. The measurement at 2.7 GHz was made with the 300-foot antenna of the National Radio Astronomy Observatory. Observations of flux density and linear polarisation at 4.9, 8.0 and 14.5 GHz were made with the University of Michigan 85-foot paraboloid using a previously described technique⁴. The measurements at 31.4 and 85.2 GHz were made with the 36-foot antenna of the NRAO as part of a millimetre wave variable-source programme being conducted by Hobbs and Dent. Included in Table 2 are a flux density measurement at 5 GHz made with the NRAO 300-foot antenna by J. W. Warner and J. H. Black (private communication) and the average of observations on two days at 90 GHz by B. J. Wills⁵.

The characteristics of the observed radiation are consistent with the synchrotron radiation process. The emission extended over a broad range of frequencies; and the source was linearly polarised during the outburst by up to 3% at both 8 and

Fig. 1 The flux density against UT date at 14.5 (×) and 8.0 (○) GHz. The arrows labelled a, b, c, d indicate the epochs of the spectra shown in Fig. 2.

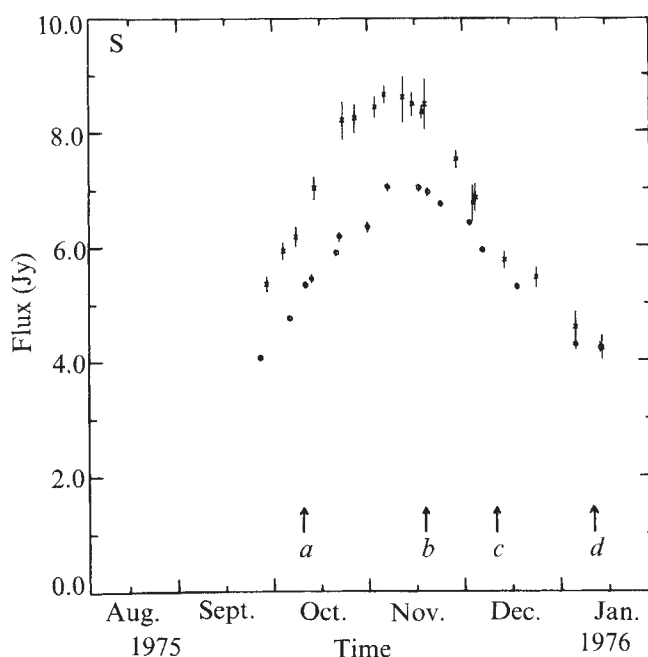
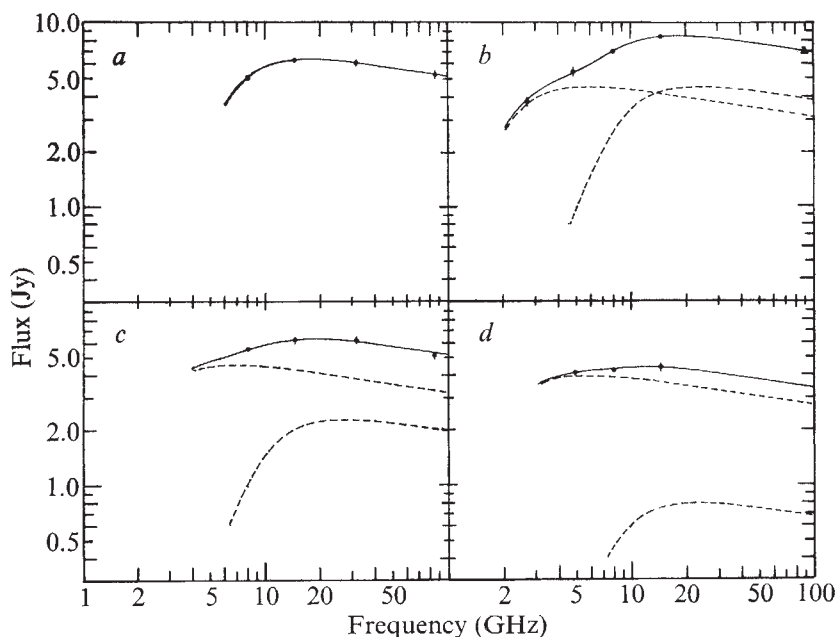


Table 1 Flux density variations with time*

Date (UT)	8.0 GHz S_v (Jy)	σ_s (Jy)	14.5 GHz S_v (Jy)	σ_s (Jy)	Date (UT)	8.0 GHz S_v (Jy)	σ_s (Jy)	14.5 GHz S_v (Jy)	σ_s (Jy)
August 1974					November 1975				
19	1.21	0.09			12			8.60	0.45
September 1975					15			8.48	0.21
27	4.05	0.06			17	7.02	0.08		
29			5.34	0.13	18			8.35	0.13
October 1975					19			8.48	0.45
4			5.93	0.16	20	6.95	0.08		
6	4.74	0.05			24	6.74	0.07		
8			6.17	0.18	29			7.52	0.16
11	5.33	0.07			December 1975				
13	5.44	0.08			3	6.41	0.06		
14			7.02	0.20	4			6.75	0.32
15			7.21	0.59	5			6.84	0.25
21	5.89	0.06			7	5.93	0.05		
22	6.18	0.09			14			5.75	0.15
23			8.20	0.34	18	5.28	0.06		
27			8.24	0.26	19			6.31	0.50
31	6.34	0.10			24			5.45	0.18
November 1975					January 1976				
3			8.43	0.19	6	4.26	0.09	4.57	0.27
6			8.64	0.16	13	4.23	0.10	4.21	0.21
7	7.04	0.08							

* Observations made with University of Michigan 85-foot antenna.

Fig. 2 Spectrum of AO 0235+164 at four epochs: *a*, October 9, 1975; *b*, November 18, 1975; *c*, December 10, 1975; and *d*, January 10, 1976. The solid curves were fitted to the data by summing the two self-absorbed homogeneous synchrotron source spectra shown as dashed lines. The values shown at 8.0 and 14.5 GHz are interpolations of the data shown in Fig. 1. In *b* the 5-GHz data point is an average of two measurements made on November 4 and November 24, 1975 and the 90-GHz data point is an average of measurements made on November 20 and November 21, 1975.



14.5 GHz and to a higher degree at optical frequencies⁴. A preliminary analysis of our linear-polarisation data indicates no significant difference between the observed polarisation position angles at 8 and 14.5 GHz, which is consistent with the hypothesis that there is no Faraday rotation within the source. The polarisation position angle at 14.5 GHz during early October 1975 was $162 \pm 8^\circ$ which agrees, within our error, with the polarisation position angle observed at optical wavelengths by Kinman⁴ on October 3, 1975. This agreement certainly suggests that the radio and optical radiation originated in regions having the same magnetic-field orientations, but the much lower degree of polarisation at 14.5 GHz ($2.2 \pm 0.6\%$) indicates that the magnetic field in the radio-emitting region is less well ordered. These polarisation data are evidence of a direct association between the optical and radio-emitting regions.

The time dependence of the radio outburst at 8.0 and 14.5 GHz is shown in Fig. 1. Before the maximum in mid November the fractional rate of increase was the same ($1.7\% \text{ d}^{-1}$) at the two frequencies. The maximum at 14.5 GHz seemed to occur on November 8 which was ~ 6 d before the apparent maximum at 8.0 GHz. Such a delay is qualitatively consistent with expanding synchrotron-source models, but the observed ratio of 1.2 for the flux densities at maxima is significantly less than the ratio of 2.0 predicted by the instantaneous injection model⁵. The observed behaviour of the flux density at the two frequencies could be explained by either a prolonged injection or an acceleration of radiating particles from August through mid November 1975 (ref. 6). The complex light curve at infrared and optical wavelengths also reached a maximum in mid November⁴ and appears to be direct evidence of just such a prolonged injection.

The observed radio spectra of the source at four epochs are displayed in Fig. 2. The most striking feature of three of the spectra is the broad low frequency turnover which cannot be fitted by a single homogeneous, self-absorbed synchrotron emitting region but which is characteristic of inhomogeneous synchrotron sources⁷. As the simplest representation of the spectrum of an inhomogeneous source, we have summed the spectra (shown as dashed lines) of two homogeneous emitting regions which become self-absorbed at different frequencies. The progressive flattening of the spectrum at < 14.5 GHz after the maximum in mid November (see Fig. 1) is consistent with the turnover progressing towards lower frequencies. This phenomenon is illustrated by the systematic decrease in the amplitude of the high frequency spectral component in Figs 2c and 2d. Above 30 GHz the source was completely transparent during the period of observation, and our data are consistent with the spectral index α remaining constant at $\alpha = -0.15$. The fact that the source was transparent before reaching maximum is inconsistent with instantaneous-injection expanding source models. It requires that the injection or acceleration of the radiating particles was prolonged and that energy was supplied to the radiating particles more rapidly than it was lost due to expansion of the emitting region. Synchrotron and inverse Compton energy losses seem to have been unimportant for particles radiating at < 100 GHz, as there was no significant steepening of the spectrum with time.

An extrapolation of the radio spectrum in mid November predicts a $\lambda = 20 \mu\text{m}$ flux density of 3 Jy compared with an observed flux of 1 Jy (ref. 4). The continuity between the radio and infrared fluxes suggests that the emission over the entire spectrum is produced by synchrotron radiation. The observed spectral index of -1.2 in the infrared and -3.8 at optical wavelengths^{2,4} indicates that the synchrotron emission cuts off in the latter spectral region.

A quantitative analysis of the observed emission places additional constraints on models for the source because of the apparent high brightness temperature implied by the rapidity of the variations. Based on the observed fractional rate of change of the flux density, the apparent radius of the source must be $\lesssim 100$ light d. Two absorption line redshift systems, with $z = 0.524$ and $z = 0.851$, have been observed in the optical spectrum⁴. The larger redshift would correspond to a cosmological distance of $\sim 6 \times 10^9$ pc. This distance and apparent physical size yields an angular diameter of 2×10^{-5} arc s and implies an apparent source brightness temperature of $T_b \approx 10^{15}$ K at 8 GHz. Such a high intensity would result in the

Table 2 Spectral data

Date (UT)	Frequency (GHz)	S_ν (Jy)	σ_ν (Jy)	Antenna
October 9, 1975	31.4	6.09	0.21	36' NRAO
	85	5.21	0.27	36' NRAO
November 5, 1975	4.9	5.47	0.32	85' Univ. Michigan
November 18, 1975	2.7	3.74	0.20	300' NRAO
November 20.5, 1975	90	6.97	0.20	36' NRAO
November 24, 1975	5.0	5.70	0.30	300' NRAO
December 10, 1975	31.4	6.24	0.18	36' NRAO
	85	5.04	0.10	36' NRAO
January 10, 1975	4.9	4.10	0.15	85' Univ. Michigan

inverse Compton process^{8,9} producing a much larger flux of infrared and shorter wavelength radiation than was observed. The observed maximum flux density in the optical B band of 3.7×10^{-3} Jy (ref. 4) can be used to set an upper limit on the radio brightness temperature¹⁰ of $\sim 10^{12}$ K at 8.0 GHz. The disparity between this upper limit and the apparent observed brightness temperature is eliminated if the source expands with a relativistic velocity^{10,11} or if it is much closer than the cosmological distances associated with the observed redshifts. An expansion velocity corresponding to a Lorentz factor of ~ 10 is sufficient to reduce the brightness temperature in the reference frame of the emitting region to $< 10^{12}$ K and would make the inverse Compton process energetically unimportant in the source. A relativistic expansion of the source would also produce a broadening in the low-frequency self absorption turnover of the spectrum¹² which is qualitatively consistent with our observed radio spectra of AO 0235+164.

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Photometric and spectroscopic observations of the BL Lacertae object AO 0235+164

BL LACERTAE objects pose, in extreme form, the question of the nature of the most energetic extragalactic sources. They exhibit rapid variability over a large dynamic range, large and variable intrinsic polarisation, and high luminosity, a combination that is very difficult to reconcile with current physical theory. In this paper, we confirm that AO 0235+164 is a BL Lacertae object with one absorption-line redshift at $z = 0.524$ and a probable one at $z = 0.852$. Photometry between 0.36 and 21 μ m and at 90 and 140 GHz showed a peak luminosity in November 1975 comparable with the most luminous QSOs.

Extensive studies in the optical and radio spectral regions have documented the variations in flux and polarisation for a number of these sources^{1,2}. The available infrared observations are far less complete, even though BL Lac-type objects emit a large, frequently dominant, portion of their energy in this spectral region. Coordinated programmes to study the behaviour of these sources in all three spectral regions are needed.

Their lack of emission lines has made it difficult even to

estimate the distances of these sources. Two absorption lines identified as the Mg II doublet redshifted by $z = 0.424$ have, however, been found in the spectrum of one of these objects (PKS0735+178) (ref. 3). In addition, BL Lac itself and some probably similar objects (1101+38, 1652+39; ref. 2) have surrounding nebulosity which shows absorption lines at redshifts of $z < 0.1$.

AO 0235+164 is a newly discovered member of this class of object. Based on the coincidence of precise optical and radio positions, it was identified by Argue, Kenworthy, and Stewart⁴ with a 19-mag, apparently stellar object. Spinrad and Smith⁵ found that the optical object had a featureless spectrum at wavelengths $> 4,400$ Å and varied between $V = 17.5$ and 19.5 during 1972-74. They reported that the object was red ($B-V \simeq 1.3$) and had an apparent faint wisp of nebulosity extending 3'' S from the stellar source. Spinrad and Smith also cited radio observations that show a spectrum rising with increasing frequency and indicate variability at 6 cm; they suggested that AO 0235+164 is a BL Lac-type object with an unusually red colour.

The outburst of AO 0235+164

We have studied the recent outburst of this source in which it reached a magnitude of $V = 14.3$. Its spectrum has a strong and rich absorption-line system at $z = 0.524$, and probably a second system at $z = 0.852$. If these redshifts are of cosmological origin, AO 0235+164 reached a peak luminosity $\geq 3 \times 10^{14} L_{\odot}$, comparable with the outputs of the most luminous QSOs. Most of this energy fell in the infrared. AO 0235+164 is one of the most rapid variables and has one of the largest amplitudes and linear polarisation in the optical region found for any QSO-like object. Extensive observations show that the shape of the infrared spectrum fluctuated during the outburst. Our radio observations supplement those reported in papers by MacLeod *et al.*²⁷ and Ladden *et al.*⁶ which provide a thorough record of the outburst in this spectral region.

On October 3, 1975, UT, AO 0235+164 was 2 mag brighter than its brightest reported by Spinrad and Smith. On the following night, it had brightened by a further 0.18 mag. Further photoelectric photometry, obtained occasionally during the outburst, was supplemented by photographic observations of the B magnitude. All of these measurements are summarised in Table 1 where they have been converted to flux densities using the calibration of Johnson⁷. The linear polarisation of AO 0235+164, measured with an unfiltered S-11 photocathode at the Kitt Peak National Observatory (KPNO) 4-m telescope, was $24.6 \pm 0.5\%$ at a position angle of 154.2° on October 4, 1975, UT, and only $6.1 \pm 1.2\%$ at a position angle of 126.4° on January 4, 1976, UT.

Measurements

The large range and rapidity of the optical variations and the high and varying polarisation are characteristic of BL Lac-type objects and confirm the classification by Spinrad and Smith. It is noteworthy that the $B-V$ colour (0.98 mag) was about the same as that found by Spinrad and Smith before the source had brightened by more than a factor of 10. The UBV colours of AO 0235+164 are similar to those of weak-lined red degenerate stars and are slightly redder than those of weak-lined subdwarfs, emphasising the importance of precise optical and radio positions for identifying objects of this type.

Infrared measurements, also summarised in Table 1, were begun on October 7, 1975, UT. It was possible to observe the source approximately every two weeks during the outburst because of a fortuitous arrangement of telescope schedules. Near maximum, AO 0235+164 was sufficiently bright that complete infrared spectra extending from 1-21 μ m could be obtained. The star α Ari was used as a flux standard and the photometry was reduced according to the calibration described by F. J. Low²⁸. Where appropriate, the measurements

Table 1 Photometric data for AO 0235+164

μm Waveband Date (UT)	0.36 <i>U</i>	0.44 <i>B</i>	0.55 <i>V</i>	1.24 <i>J</i>	1.6 <i>H</i>	2.2 <i>K</i>	3.6 <i>L</i>	4.85 <i>M</i>	8.4	10.6 <i>N</i>	11	12.6	19	21 <i>Q</i>
1975														
Oct. 3	0.40*	1.12	2.38											
Oct. 4	0.47*	1.34	2.81											
Oct. 7										144(20)‡				
Oct. 8		1.88(18)†		30(3)‡		72	128							
Oct. 10		1.90(16)§												
Oct. 11		1.97(12)§												
Oct. 12		1.75(15)§												
Oct. 26				26*	36.5	67	127	280(35)	290(50)		330(40)	370(70)		
Oct. 27	0.76"	2.01	4.03	27*	39	70								
Oct. 28	0.67"	1.77	3.44											
Nov. 8								160‡(50)		500				1,060(190)
Nov. 9								200(40)		525				660(120)
Nov. 11				36‡	53	83	150	240(25)						
Nov. 14				48‡	66	108	182							
Nov. 24	0.57*	3.52(37)§	3.26											
Nov. 27		1.54												
Dec. 1				24*	29	53	105	230(55)	280(50)		300(40)		430(90)	650(260)
Dec. 2		1.94(40)†		23‡(1.4)	37	65‡(10)	108			262(30)				860(170)
Dec. 3		1.54(11)†				59				200(25)				
Dec. 18				14.6●	21.5	36	62							
Dec. 19						31"				237(40)●				
1976														
Jan. 3	0.11*	0.27	0.59											
Jan. 4		0.28*												

All flux densities are given in units of 0.001 Jy; when errors exceed 5%, they are given in parentheses.

*KPNO 4-m telescope.

†KPNO 0.3-m Schmidt telescope.

‡NASA 1.54-m telescope.

§KPNO 0.4-m telescope (Number 3).

"KPNO 2.1-m telescope.

●Steward Observatory 2.25-m telescope.

The photographic observations on the KPNO 0.3-m and 0.4-m telescopes were made by C. T. Mahaffey using Kodak IIa-o emulsion and a Schott GG-385 filter. The photometric magnitudes of the comparison stars that were used to calibrate these plates (together with their coordinates relative to AO 0235+164) are:

(A) $V = 15.00$; $B - V = 1.21$ (13" E; 21" N).

(B) $V = 13.04$; $B - V = 0.58$ (32" W; 32" S).

(C) $V = 16.77$; $B - V = 1.49$ (7" W; 78" N).

have been corrected for the effects of the bandpass filter on the apparent fluxes⁶.

Discussion of photometry

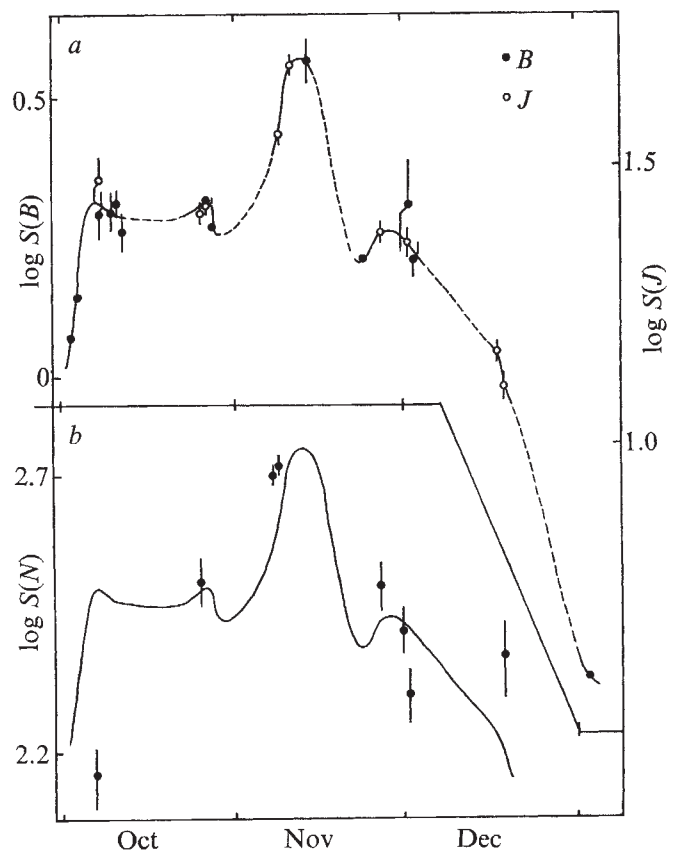
The infrared and visual light curves of AO 0235+164 are illustrated in Fig. 1. The behaviour of the source was roughly similar to that in a previous outburst during 1939–40, seen on plates in the collection of Harvard College Observatory⁸. The light curve is also similar to those of other BL Lac-type objects during violent outbursts, although AO 0235+164 is one of the most rapidly variable of this class.

From Fig. 1a it can be seen that the variations in the blue and near infrared are the same within the errors. The 10- μm observations, shown in Fig. 1b, give about the same amplitude as the visual curve, but appear to show different short time scale behaviour. The $U-B$ colour also varied slightly during the outburst. The radio flux reached a maximum at about the same time as in the optical and infrared, but the radio amplitude was considerably smaller⁶. From a comparison with the radio variations, because the source was relatively faint during our first observations, and because of the steep rise in the first part of the light curve, we believe that our first measurements were made during the initial phase of the outburst.

Variability of up to 30% on time scales of 1 or 2 d was seen both in the visible and infrared. Although we did not obtain sufficient simultaneous photometry in these two wavelength regions to prove that these fluctuations always occur simultaneously, that conclusion is indicated, since first, the infrared increase between November 9 and 11 was accompanied by an easily noticeable brightening as viewed through the telescope, and second, the spectral shape in each region implies that any variations should be simultaneous. Rapid variations of such large amplitude were not seen in the radio flux⁶.

The photometric spectrum of AO 0235+164 on six occasions during the outburst is shown in Fig. 2. Where simultaneous measurements at all wavelengths were not available, the necessary extrapolations were based on Fig. 1a. The average correction for galactic extinction at the latitude of AO 0235+164 (-39°) is only 0.01 mag in $U-B$ and 0.02 mag in $B-V$

Fig. 1 Light curves of AO 0235+164 with flux densities in mJy. The upper panel (a) is for B (0.44 μm) and J (1.25 μm) except that the last point of J was obtained from a measurement at 2.2 μm and the mean [1.25 μm –2.2 μm] colour. The lower panel (b) is for N (10 μm); some narrow-band measurements near this wavelength have been combined to increase the statistical weight. The curve is the same as that used in (a).



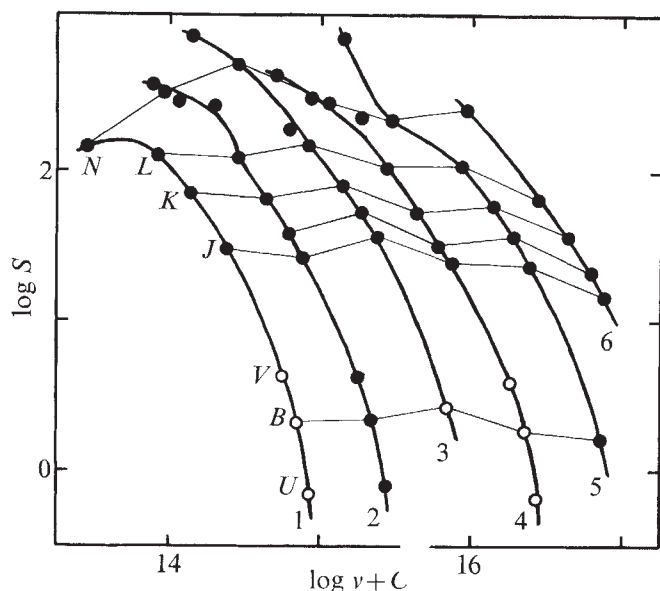


Fig. 2 Photometric spectra of AO 0235+164 with flux densities in mJy. Thick lines join measurements taken at the same time while thin lines connect measurements at the same frequency. For clarity, successive epochs are displaced sideways. The 1975 UT dates and values of the constant C are: curve 1—Oct. 7–8, $C = 0$; curve 2—Oct. 26–27, $C = 0.5$; curve 3—Nov. 8–9, $C = 1.0$; curve 4—Nov. 27, $C = 1.5$; curve 5—Dec. 1–2, $C = 2.0$; curve 6—Dec. 18–19, $C = 2.5$. $\circ$, points that were extrapolated according to Fig. 1a by > 24 h.

(ref. 10), and there is no indication of strong localised extinction near the source. The $(10.6\text{--}0.44\text{ }\mu\text{m})$ and $(0.55\text{--}0.36\text{ }\mu\text{m})$ spectral indices varied over the ranges -1.49 ± 0.14 and -4.14 ± 0.12 , respectively. Although the spectra of most BL Lac-type objects are slightly curved between 0.4 and $10\text{ }\mu\text{m}$ (ref. 11), that of AO 0235+164 has substantially greater curvature and is steeper than is typical.

The spectra in Fig. 2 show the effects of the different short term variability beyond $4\text{ }\mu\text{m}$. Although these effects are apparent throughout the outburst, they are particularly striking at the beginning when the spectrum appears to turn over near $10\text{ }\mu\text{m}$. Another explanation — that the source brightened by a factor ≥ 2 between October 7 (when the $10\text{ }\mu\text{m}$ data were obtained) and October 8 (when the other measurements were made) — seems unlikely because there was no noticeable brightening observed visually between these two dates, and fluctuations of the required amplitude and speed were not seen at any other time. Instead, it seems that the flux rose

more slowly at $10\text{ }\mu\text{m}$ than at shorter wavelengths.

The 90 and 140 GHz measurements of AO 0235+164, summarised in Table 2, together with other mm-wave flux densities, indicate a maximum which occurred at about the same time as in the optical and infrared, but they show a slower decay. The radio outburst is discussed in detail in refs 6 and 27.

Photographic results

A deep plate in the yellow-red (Eastman Kodak 127-04 emulsion and Schott GG-495 filter) was taken at the prime focus of the KPNO 4-m telescope on January 4, 1976, UT, when the object was at $B \approx 18.0$. The image of AO 0235+164 on this plate, and that of a star of comparable brightness ($6''\text{W}$ and $187''\text{N}$), were scanned on the PDS microphotometer at KPNO using a square raster scan of side $13''$ and a scanning aperture of $0.15''$. These data were displayed on the COMTAL television monitor of the KPNO Interactive Picture Analysis system¹². Identical optimum adjustments were made to show the faint extensions of the images; photographs of this display

Fig. 3 Photographs of a television reconstruction of the images of *a*, AO 0235+164 and *b*, a nearby star to show the southerly extension of the former. North is to the top and E to the left, and each square has a side of $13''$.

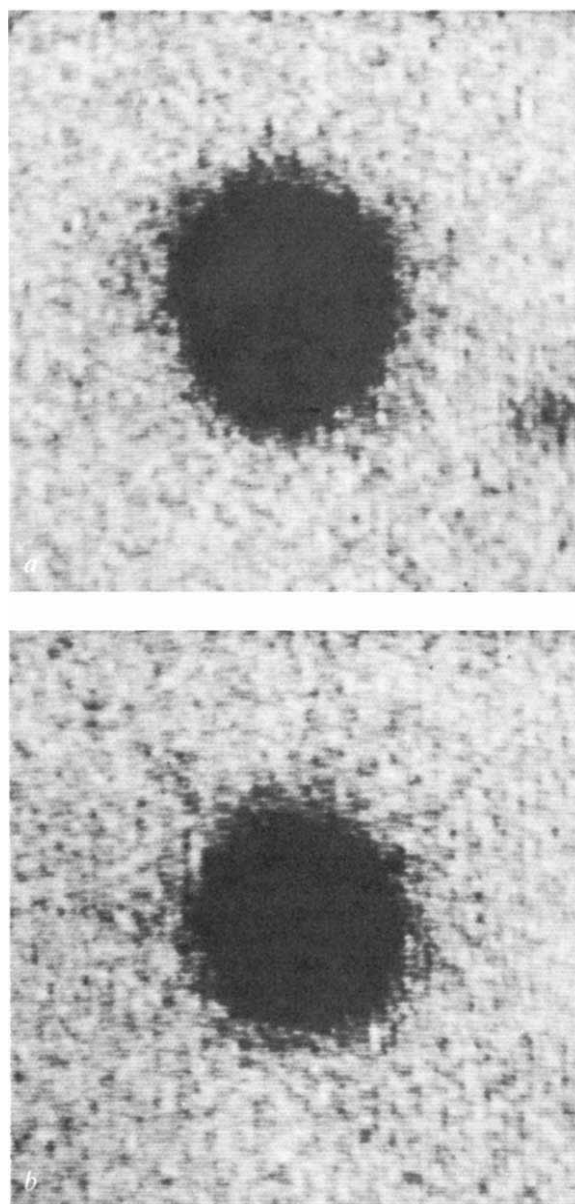


Table 2 High frequency radio observations of AO 0235+164

Date (UT) 1975	Flux density (Jy)	Frequency (GHz)	Telescope*
Nov. 20	6.9 ± 0.3	90	A
Nov. 21	7.2 ± 0.3	90	A
Nov. 22	6.6 ± 0.3	90	A
Nov. 23	7.0 ± 0.3	90	A
Nov. 28	6.1 ± 1.2	140	B†
Dec. 1	3.4 ± 1.4	140	B†
Dec. 18‡	5.4 ± 0.4	90	A

*A = 11-m telescope of the National Radio Astronomy Observatory at KPNO which is operated by Associated Universities, Inc., under contract with the National Science Foundation.

B = 4.9-m telescope of the mm-Observatory operated by the Electrical Engineering Laboratory of the University of Texas at Austin, with support from NASA, NSF and the McDonald Observatory.

†These observations were made with assistance from A. Uomoto.

‡This result was kindly made available by Dr E. K. Conklin.

are shown in Fig. 3. The southerly extension of the image of AO 0235+164 found by Spinrad and Smith is confirmed, but its maximum extent ($\sim 3''$) is no greater than that reported by them, even though the KPNO plate is deeper. A nebula or galaxy associated with the source is an exciting possibility, but our observation is also consistent with the existence of a faint, superposed star.

Absorption-line spectrum

A spectrogram of AO 0235+164, obtained under poor conditions on November 25 with the Steward Observatory 2.25-m telescope, showed an absorption feature which was identified as the Mg II doublet. Spectrograms were independently obtained between December 3 and 8 (UT) using a two-stage image-tube spectrograph on the 2.7-m telescope of McDonald Observatory. The reciprocal dispersion was $110 \text{ \AA mm}^{-1}$ and baked IIIa-J emulsion was used, giving a resolution of $\sim 3 \text{ \AA}$ over the spectral range 3,500–5,500 $\text{\AA}$. As an illustration, a density tracing of one of these spectrograms is shown in Fig. 4. Some of the features are clearer on other spectrograms; in particular, the lines near 3,600 $\text{\AA}$ are seen most clearly on a spectrogram optimised for that region. The existence of some of the weak lines is supported by their detection on a number of spectrograms.

The principal feature in the spectrum is a well resolved pair of deep, sharp absorption lines which we identify as the Mg II ($\lambda = 2,795.5$ and $2,802.7 \text{ \AA}$) doublet at a redshift $z = 0.5242 \pm 0.0002$; the width of these lines is $< 2 \text{ \AA}$ in the rest frame. There are many other absorption lines, identified as Mg I, Fe II, and Mn II; these are listed in Table 3, together with the number of spectrograms on which each line was measured.

All the lines listed as strong in Table 3 were also seen in PHL938 (ref. 13) and in PKS1331+170 (ref. 14) and are those predicted by Bahcall¹⁵ to be the most easily detectable in QSOs within the range of rest-wavelengths covered by our spectrograms. The Mg I and Mn II lines have not been found previously in QSO spectra, but their identification is supported by a comparison with the ultraviolet spectrum of ζ Oph (ref. 16, and E. M. Burbidge, *et al.*, ref. 29). In fact, all the lines which occur in the appropriate wavelength region for the $z = 0.524$ system and which have equivalent widths $> 30 \text{ m\AA}$ in the spectrum of this star are also seen in the spectrum of AO 0235+164. The rich absorption-line spectrum at $z = 0.524$ removes any doubt about the correctness of the identification of the Mg II doublet in this object and, by analogy, in PKS0735+178.

On three of the spectrograms there is a pair of absorption

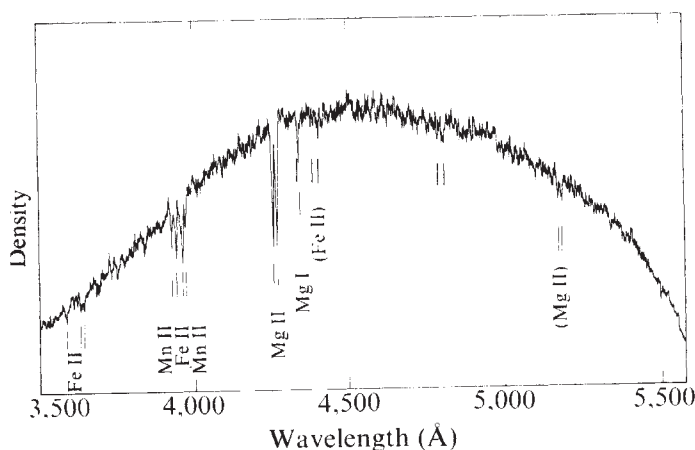


Fig. 4 A density tracing of a spectrogram of AO 0235+164. Symbols without parentheses denote line identifications in the $z = 0.524$ redshift system, while symbols within parentheses indicate lines in the $z = 0.851$ system. The vertical lines without symbols show the expected positions that weaker members of the $z = 0.524$ system would have with a redshift of $z = 0.851$.

Table 3 Absorption lines in AO 0235+164

λ_{obs}	Strength*	Identification	λ_0	Redshift	No. of spectrograms measured
3572.1	S	Fe II	2343.5	0.5243	2
3599.2?	W				
3618.8	S	Fe II	2373.7	0.5245	2
3631.4	S	Fe II	2382.0	0.5245	2
3927.6	W	Mn II	2576.1	0.5246	3
3941.2	S	Fe II	2585.9	0.5241	3
3953.7	W	Mn II	2593.7	0.5243	2
3963.0	S	Fe II	2599.4	0.5246	3
3972.2	W	Mn II	2605.7	0.5244	3
4260.6	S	Mg II	2795.5	0.5241	3
4271.9	S	Mg II	2802.7	0.5242	3
4347.0	M	Mg I	2852.1	0.5241	3
4412.2	W	Fe II	2382.0	0.8523	3
4432.2?	W				
5176.0	W	Mg II	2795.5	0.8515	3
5189.7	W	Mg II	2802.7	0.8517	2

*S, M, W = strong, medium, weak absorption relative to the continuum.

lines near 5,176 and 5,190 $\text{\AA}$ which cannot be readily identified if their redshift is 0.5242. Figure 5a shows the averaged density scan of these spectrograms; we suggest that the lines are the Mg II doublet at a redshift of 0.8516 ± 0.0007 . Since these Mg II lines are only about one-quarter of their strength in the $z = 0.524$ system, even the strongest Fe II lines should be only barely detectable in the 0.852 system. A weak line measured at $4,412.2 \pm 0.7 \text{ \AA}$ on three spectrograms corresponds to Fe II ($\lambda = 2,382 \text{ \AA}$) and the composite tracing shown in Fig. 5b lends further support to the reality of this line. The dip near 4,811 $\text{\AA}$ in Fig. 4 corresponds to the expected position of Fe II ($\lambda = 2,599 \text{ \AA}$) in the $z = 0.852$ system; it was not seen on the other three spectrograms, but they were less densely exposed in this wavelength region. The Fe II ($\lambda = 2,343 \text{ \AA}$) line would occur only 8 $\text{\AA}$ to the blue of the Mg I line (see Fig. 5b) and would therefore be difficult to detect. Since the redshifts of emission-line systems in QSOs are usually at least as large as the largest absorption-line redshift, the continuum of AO 0235+164 is probably at $z \geq 0.852$. Our spectroscopic results are in good agreement with those of Burbidge *et al.*²⁹, although our measurements extend further into the ultraviolet.

Emission lines

AO 0235+164 showed no emission lines on any of our spectrograms, including one exposed between 3,700 and 7,000 $\text{\AA}$ at a reciprocal dispersion of $240 \text{ \AA mm}^{-1}$, which should have been particularly sensitive to broad lines. The presence of strong absorption lines without emission lines is very uncommon among known QSOs, a situation that may be partly a result of selection effects. Schmidt¹⁷ noted the rare case of 4C 28.25, a QSO with an absorption-line system consisting of the Mg II doublet and the Fe II lines $\lambda = 2,382 \text{ \AA}$, $\lambda = 2,586 \text{ \AA}$, and $\lambda = 2,599 \text{ \AA}$ with a redshift of $z = 0.8907$. 4C 28.25 also has weak emission features identified with C III and Mg II with a redshift of $z = 0.899$. 4C 28.25 is, therefore, intermediate between an emission-line QSO and objects like AO 0235+164. We also note that incipient emission with a redshift close to that of the absorption spectrum could distort the profiles of the absorption lines in AO 0235+164 and invalidate estimates of multiplet ratios.

Discussion

The relative line strengths in the $z = 0.524$ system may be difficult to reconcile with strictly solar abundance ratios in the gas and simple density and velocity distributions. The arguments in this paragraph are supported by the detection of structure in the 21-cm absorption line at $z = 0.52385$ ³⁰. For example, explaining the relative strengths of the Mg II and Fe II lines by having them form lower on the curve of growth than in ζ Oph (E. M. Burbidge, *et al.*, ref. 29)—assuming a relatively

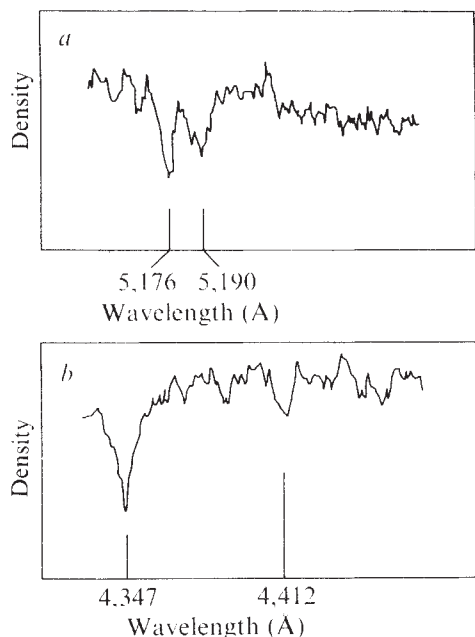


Fig. 5 Composite density tracings of the spectrograms to show the features in the $z = 0.852$ redshift system: *a*, the region of the Mg II doublet (5,176 and 5,190 Å) from three spectrograms and *b*, the region of the Fe II at $\lambda = 2,382\text{Å}$ (4,412 Å) from four spectrograms.

low column density and high velocity dispersion—would require an overabundance of Mn and would be hard to reconcile with the relative strengths of the Fe II lines at 3,619, 3,631, 3,941, and 3,963 Å. If the relative strengths of the Mg II and Fe II lines are a result of a very high column density and small velocity dispersion¹⁸, the Fe II lines should be more strongly saturated than indicated by the equivalent widths given by Burbidge *et al.*

High column densities might also result in heavy extinction, particularly if the absorption lines arise in an intervening galaxy with an absorption line-extinction ratio similar to that found in interstellar clouds in our own Galaxy. Extinction could contribute to the large curvature of the spectrum of AO 0235+164. The *UBV* colours of AO 0235+164 would be consistent with those of a typical BL Lacertae object with an additional reddening of ~ 0.5 mag in its *B-V* colour. Mg II column densities of as much as 10^{+17} cm^{-2} (a possibility suggested by Wolfe and Burbidge¹⁸ for PKS0735+178 for which the doublet is slightly weaker) lead, however, to enormous values of the reddening, if the extinction process is similar to that which applies to the interstellar material in our own Galaxy. For example, ζ Oph, with a Mg II column density of only 10^{15} cm^{-2} (ref. 15) has $E_{B-V} \simeq 0.3$. Also, PKS0735+178 does not appear to be significantly reddened. An intrinsic ultraviolet cutoff in the continuum provides an attractive explanation for the lack of emission lines in BL Lacertae-type sources. AO 0235+164 may be the first such object found at sufficiently high redshift that a cutoff is detectable through *UBV* photometry.

Most of the detected energy from AO 0235+164 was in the infrared and its peak luminosity was $\geq 3 \times 10^{14} L_{\odot}$, if it is at the Euclidean distance implied by the redshift of $z \geq 0.852$ ($v/c = 0.55$) and a Hubble constant of $55\text{ km s}^{-1}\text{ Mpc}^{-1}$. The total flux density change is 2 orders of magnitude, but its outbursts seem to be relatively infrequent and of sufficiently short duration that the total energy emitted at the lower level of activity is comparable with that released during outbursts. The peak luminosity of AO 0235+164 was $\sim 10^5$ times greater than the luminosity measured for the nuclear source in B2 1101+38²⁰. This source has similar properties in other respects: a radio spectrum that is flat or rises with increasing frequency, past large amplitude variability, large and variable intrinsic

polarisation, and an absence of emission lines^{20,21}. Thus, a single phenomenon seems to operate at power levels ranging from those typical of moderately active galactic nuclei up to those associated with the most luminous QSOs.

Constraints on models

It is generally believed that the radio emission from sources of this type is some form of synchrotron radiation²¹. AO 0235+164 is an outstanding example where the cosmological interpretation of the redshift and the time scale of the variability are very difficult to reconcile with this hypothesis without invoking highly relativistic motion or a special geometry²².

Attempts to explain the optical and infrared emission as synchrotron radiation encounter even greater difficulties. Except for B2 1101+38 (ref. 23), these sources have not been detected in the X-ray region^{24,25} (the pre-outburst Uhuru upper limit for AO 0235+164 is $1.7 \times 10^{-10}\text{ erg cm}^{-2}\text{ s}^{-1}$). To reconcile the lack of Compton-scattered X-ray fluxes with the high surface brightness implied by the rapid fluctuations, very large magnetic fields and very special geometries are required^{26,2}. Further X-ray observations during the outbursts of these sources are urgently needed.

Any plausible model for optical synchrotron emission would probably require a sufficiently large magnetic field so that synchrotron self-absorption would occur in the infrared¹¹. The behaviour of the spectrum of AO 0235+164 beyond $4\text{ }\mu\text{m}$ and the differences between the radio and optical-infrared light curves could be taken as evidence for self-absorption.

It has not yet been demonstrated, however, that self-consistent models can be constructed along these lines, and our observations of AO 0235+164 strengthen the difficult constraints on solutions to the problem. It is possible that some other, not yet understood emission mechanism is operating, or that these sources are not at the cosmological distances indicated by their redshifts.

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Messenger RNA for the coat protein of tobacco mosaic virus

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TMV RNA is not an efficient template for translation of the viral coat protein, in spite of containing nucleotide sequences coding for the protein. Efficient translation requires the prior synthesis within infected cells of a smaller RNA carrying only a portion of the information encoded in the whole genome.

TOBACCO mosaic virus (TMV) consists of an infectious single-stranded RNA molecule 6,400 nucleotides long, encapsidated by 2,100 identical coat protein molecules each with a molecular weight of 17,500 (ref. 1). This packaged RNA (virion RNA) is the sense strand for the translation of coat protein, as was first shown by patterns of mutagenesis^{2,3} and more recently by direct nucleotide sequence analysis⁴. The coat protein gene must consist of at least 480 residues of virion RNA. Thus less than 10% of the RNA is accounted for by the coat cistron.

As would be expected, the RNA acts as a good message in cell-free translation systems from bacteria⁵, plants⁶ and animals⁷, but analysis of the products of such incubations has repeatedly shown that coat protein is not the major product, and careful studies with the *E. coli* cell-free system eventually proved that ribosomes never passed over the coat protein cistron in the correct phase^{8,9}. This might be due to general incompatibility between eukaryotic and prokaryotic mRNA and ribosomes, but studies of the translation of TMV RNA in various eukaryotic protein synthesising systems have given similarly negative results, thus suggesting that translation of coat protein is not straightforward, and raising the problem of how the coding sequences known to be present on the RNA actually are expressed. In the wheat germ cell-free system, for example, the major products are much larger than the coat protein and also very heterogeneous. Nevertheless Efron and Marcus⁶ detected labelled coat protein peptides in products which migrated with authentic coat protein on gel filtration although they represented only a very minor fraction of the total incorporation. In contrast, when Roberts *et al.*¹⁰ used

RNA from the TMV mutant Ni 568, they failed to detect significant levels of product with the mobility of coat protein on acrylamide gels. They did, however, find a coat protein peptide among the products of their incubation, at very low yield, and it was suggested¹¹ that the coat protein is made as part of a large precursor which is processed by proteolysis to yield mature coat, as in the case of picornaviruses. This idea is inconsistent with the data of Efron and Marcus, who failed to find any coat protein peptides in material larger than coat protein.

We have used two other eukaryotic systems to translate TMV RNA, and have found that it directs the synthesis of discrete polypeptides, which simplifies interpretation (ref. 7 and our unpublished results). In *Xenopus laevis* oocytes⁷ and in reticulocyte lysates, the products directed by TMV RNA are two large polypeptides with molecular weights of 140,000 and 165,000; the first corresponds to the largest product of the wheat germ system. We have never detected the synthesis of coat protein. The 140,000 polypeptide does not contain coat protein sequences detectable by tryptic peptide mapping⁷ and, given the results reported here, it does not seem likely that the coat is contained within the 165,000 molecular weight polypeptide either. We believe that these proteins are authentic translation products of cistrons in TMV RNA other than the coat cistron, for similar sized proteins are made in TMV-infected leaves and protoplasts^{12,13}. In synchronously infected protoplasts, these two proteins are readily detectable about 5 h after infection and there is a further lag of about 4 h before coat protein synthesis begins. Late after infection the synthesis of the two large proteins declines, but coat protein synthesis continues at very high levels. It is difficult to interpret these findings, particularly the latter observation, in terms of a precursor-product relationship between the largest proteins and the coat protein.

It thus seems that there is some temporal and quantitative control of gene expression in TMV, as has been found in essentially all RNA viruses (with the exception perhaps of the picornaviruses), and that most of the extra RNA in TMV which does not code for coat protein codes for the proteins of molecular weight 140,000 and 165,000. If this is correct, and cell-free systems and *Xenopus* oocytes make essentially only 'early' TMV proteins, then how is the coat protein made? We extracted TMV-infected tobacco leaves to see if we could find and characterise the mRNA for

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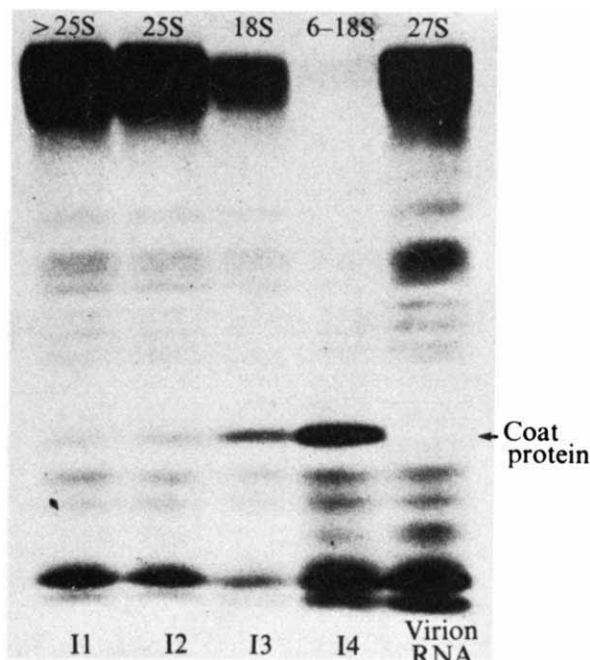
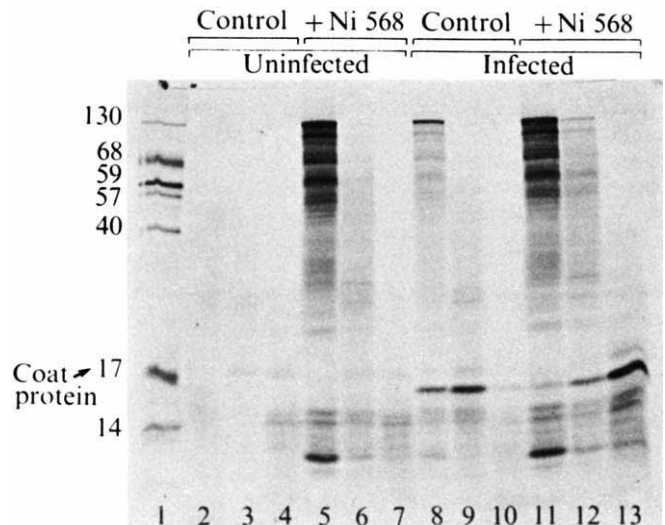


Fig. 1a. Translation of RNA of different size classes from tobacco leaves infected with TMV. Tobacco leaves, infected 7 d previously with TMV vulgare, were washed in water, ground in liquid nitrogen and extracted in an equal mixture of water-saturated phenol and buffer A (10 mM EDTA, 1% SDS, 50 mM Tris-Cl, pH 8.8) at 65 °C. The aqueous phase was extracted four more times with phenol, made 0.1 M in NaCl and precipitated with 2 volumes of ethanol. The precipitate was taken up in 1 mM EDTA, 0.5% SDS; warmed for 1 min at 65 °C, and spun at 10,000g for 30 min at 4 °C to remove undissolved material. The supernatant was layered over 15–40% (w/v) sucrose gradients in 0.1% SDS, 1 mM EDTA, 20 mM Na acetate, pH 5.0, and spun in a Beckman SW 27 rotor for 18 h at 27,000 r.p.m. at 15 °C. The gradients were pumped through a recording spectrophotometer and fractions were pooled as follows: I1 > 25S; I2 ~ 25S; I3 ~ 18S; I4 18S–6S. The RNA fractions were reprecipitated from 0.2 M Na acetate with 3 volumes of ethanol. The precipitates were taken up in water, and stored in liquid nitrogen, except for fraction I4 which was diluted in 1 mM EDTA, 1% SDS, 2 M LiCl and precipitated overnight at 4 °C (ref. 45). The precipitate was

coat protein, since large amounts of coat protein are made in infected cells, and thus some functional messenger of high efficiency clearly exists. This could in theory be the virion RNA complexed with some control protein, but there were indications from earlier work that the actual messenger might be a smaller RNA^{14,15}. This could explain the marked discrepancy between the apparently faithful synthesis of the 140,000 and 165,000 molecular weight polypeptides in *in vitro* systems, and the total lack of coat protein synthesis in the same systems when programmed with virion RNA, implying that the coat cistron in virion RNA is repressed.

Preliminary experiments

RNA was extracted from tobacco leaves which had been systematically infected with TMV 7 d previously. They were frozen and pulverised in liquid nitrogen before extraction in hot phenol–sodium dodecyl sulphate (SDS). After several phenol extractions, RNA was precipitated with ethanol, and after resuspension in aqueous buffer, fractionated on sucrose gradients. Fractions from the gradients were pooled according to their sedimentation rate and recovered by ethanol precipitation. The slowest sedimenting fraction was



recovered by centrifugation at 10,000g for 10 min, and taken up in water before ethanol precipitation as above. Each fraction, and RNA extracted from TMV virions, was tested for template activity in a cell-free wheat germ system prepared and incubated as described by Roberts and Paterson¹⁶, except that the ionic conditions in the incubation were: 80 mM KCl, 1.5 mM MgCl₂, 0.8 mM spermidine, 20 mM HEPES, pH 7.2. The labelled amino acid was L-³H-leucine (20 Ci mmol⁻¹, 10 μM). Reactions were stopped by incubation with pancreatic RNase (100 μg ml⁻¹) for 15 min at 30 °C. Four volumes of SDS gel sample buffer were added¹⁷ and the samples were analysed on 20% SDS-acrylamide gels. After fixing and staining, the gels were impregnated with PPO according to Bonner and Laskey¹⁸ and exposed to Kodak RP Royal X-ray film at -70 °C. The fluorogram shows the products directed by fractions I1–I4, and virion RNA. All RNAs were present at 200 μg ml⁻¹ in the incubations. A control without added messenger showed no detectable blackening of the film. **b.** Control for degradation of RNA during extraction. RNA was prepared and fractionated from either uninfected leaves (tracks 2–7) or TMV vulgare-infected leaves (tracks 8–13) as described in (a), except that 1 mg of purified Ni 568 virus was added per g wet weight of ground leaves to half of each leaf sample just before phenol extraction (tracks 5–7 and 11–13). The RNA was fractionated by size and the three larger size classes were tested for their template activity as described above, except that products were analysed on a 15% SDS–polyacrylamide gel. The markers in track 1 are β galactosidase, bovine serum albumin, pyruvate kinase, glucose-6-phosphatase, creatine kinase, TMV coat protein and lysozyme, all labelled with ¹⁴C-iodoacetic acid.

very heavily contaminated with carbohydrate, and was further purified by precipitation with 2M LiCl at 2 °C overnight. Each fraction, as well as corresponding fractions from uninfected plants, was added to cell-free extracts of wheat germ, and incubated essentially according to Roberts and Paterson¹⁶. The incubations were analysed by SDS–polyacrylamide gel electrophoresis on slabs¹⁷, followed by fluorography¹⁸ to detect ³H-labelled polypeptides. Figure 1a shows the result of one such experiment. A strongly radioactive band with the same electrophoretic mobility as coat protein was made only in incubations programmed with RNA from TMV-infected leaves, and not in incubations programmed with virion RNA. Furthermore, maximal synthesis occurred when the smallest RNA fractions were used, suggesting that the active template was an RNA molecule considerably smaller than intact TMV RNA.

An obvious objection to the straightforward interpretation that a small RNA molecule is the natural mRNA for TMV coat protein is that it might have arisen by degradation of virion RNA during extraction of the leaves. We sought to exclude this possibility by the experiment shown in Fig. 1b. The mutant TMV strain Ni 568 contains a single methionine residue in its coat protein², unlike the wild-type

strain vulgare which contains no methionine. We therefore repeated the extraction of RNA from leaves infected with TMV vulgare, but added a large amount of purified TMV strain Ni 568 virus just before the phenol extraction. After extraction and fractionation of the RNA as before, the fractions were tested in the wheat germ system using either ^{35}S -methionine or ^3H -leucine as label in parallel incubations. The products were analysed on slab gels as before; the results of the incubations with ^3H -leucine as label are shown in Fig. 1b. No coat protein-like products were seen in RNA from uninfected plants, whether or not TMV Ni 568 had been added during extraction. In contrast, such a protein was synthesised when RNA from infected plants was used, but it was labelled only with leucine, and not with methionine. This shows that RNA from the added virions is not converted into a form capable of being translated into the putative coat protein by degradation or modification during extraction of the RNA from leaves. The experiment also shows that uninfected leaves do not contain detectable mRNA for this protein. It should be noted that since typical virion RNA-directed products are observed in the track corresponding to the uninfected plants to which Ni 568 RNA had been added, this RNA was extracted

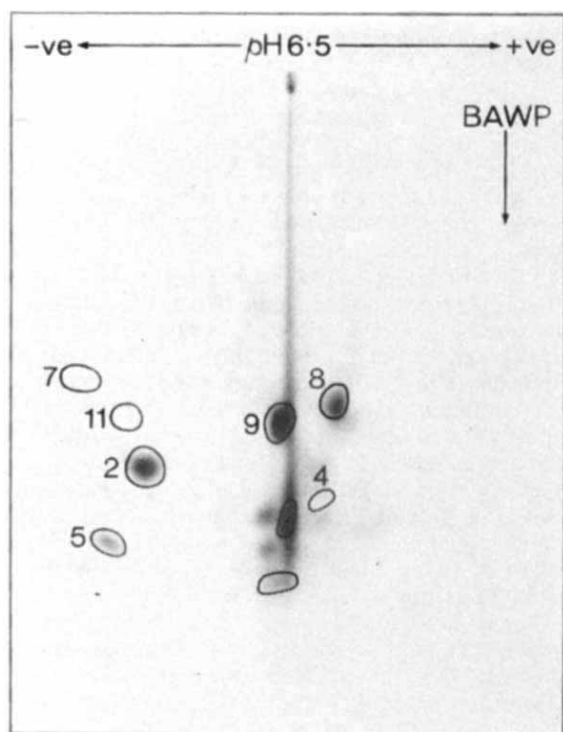


Fig. 2 Fingerprint of putative coat protein synthesised *in vitro*. Fraction 14 RNA from infected leaves was injected into 20 *Xenopus* oocytes, which was incubated in medium containing 20 μCi of $\text{L-}^{14}\text{C}$ -valine (280 μCi μmol^{-1}) for 24 h. They were homogenised as described previously⁷ with 100 μg of authentic TMV vulgare coat protein. The homogenate was centrifuged, and the supernatant fractionated by electrophoresis on an 18% SDS-polyacrylamide gel. The gel was stained with Coomassie blue, dried, and autoradiographed. The stained coat protein band, which was heavily labelled, was cut into small pieces. 2 ml of 10 mM Tris-Cl, pH 7.0, 0.1% 2-mercaptoethanol, 2% SDS were added, and the suspension was incubated for 10 min at 95 °C and for 18 h at 37 °C. The bits of paper and gel were filtered off, and the protein was recovered from the filtrate by precipitation with trichloroacetic acid (TCA). It was oxidised and digested with trypsin⁷ and the soluble peptides were separated by electrophoresis at pH 6.5, 20 V cm^{-2} on a 20-cm² Silica gel G thin layer (250 μm) (Anachem)⁴⁶, followed by chromatography in butanol-acetic acid-water-pyridine (30:6:24:20) at right angles to the first dimension. After autoradiography, the carrier TMV coat protein peptides were stained with fluorescamine⁴⁷. The stained peptides (outlined) are numbered from the N-terminus; peptides 4, 7 and 11 do not contain valine.

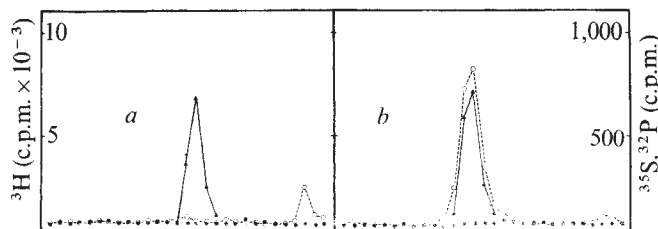


Fig. 3 Assembly of the *in vitro* product. Four cell-free incubations using the wheat germ system programmed with either fraction 14 RNA from TMV-infected leaves or 27S virion RNA, were labelled with ^{35}S -methionine or ^3H -leucine. After incubation for 1 h, the ^3H - and ^{35}S -labelled samples corresponding to each template were mixed together, and 1.8 mg of TMV coat protein was added to both mixtures. The two mixtures were dialysed against 10 mM Tris-Cl, pH 8.0, and passed over 0.5-ml columns of DE 52 cellulose equilibrated with the same buffer. After washing with buffer, the coat protein was eluted with 0.2 M KCl, 10 mM Tris-Cl, pH 8.0. This material was dialysed against 10 mM Na acetate, pH 5.0, to form the helix aggregate³¹, which was collected by centrifugation at 100,000g for 1.5 h, suspended in Na phosphate buffer, pH 7.0, ionic strength 0.1, made 5 mg ml^{-1} in coat protein by a further addition of carrier, and dialysed overnight at 4 °C against the same buffer. The sample was then incubated at 20 °C for 18 h to form disks, at which time TMV RNA (1 mg ml^{-1}) was added, and incubation continued for 10 min at 20 °C. Uncoated RNA was digested with T1 RNase (20 μg ml^{-1}) for 1 h at 20 °C, and the partially reassembled virions were purified by two cycles of preparative CsCl density gradient centrifugation, using added ^{32}P -labelled TMV as marker, in the Beckman SW 50 rotor (CsCl, 1.33 g ml^{-1} , 32 h at 35,000 r.p.m.). The fractions containing marker virus were analysed finally in 8-ml CsCl gradients in the 50 angle head rotor, spun at 40,000 r.p.m. for 24 h. a, Result of attempted assembly using the translation product specified by 27S virion RNA; b, that using incubations programmed with fraction 14 RNA. Overall recovery of radioactivity from the ^3H -labelled incubation programmed with coat mRNA in the peak of CsCl-banded reassembled virus was about 10% while essentially no radioactivity from any other incubation survived to the final analysis. $\blacktriangle$, ^{32}P radioactivity; $\circ$, ^3H radioactivity; $\bullet$, ^{35}S radioactivity.

efficiently. Furthermore, the amount of products formed suggested that we had added about the same amount of virion RNA as was naturally present in the infected leaves (compare track 5 with track 8 in Fig. 1b). We are therefore confident that the RNA which promotes the synthesis of the putative coat protein is not an artefact of degradation on phenol extraction.

Characterisation of the product

The new band programmed by RNA from infected leaves has the same mobility on SDS gels as authentic TMV coat protein, and it lacks methionine. We also found that histidine failed to label the gel band, which would be expected of coat protein, which lacks both methionine and histidine.

When the RNA from TMV-infected leaves was injected into *Xenopus* oocytes, it directed synthesis of a similar band. The yields were higher, and the background lower than in the wheat germ system, and we therefore used oocyte synthesised material to make a tryptic peptide map (Fig. 2). There is excellent correspondence between the *in vitro* synthesised peptides, labelled with ^{14}C -valine, and the fluorescamine-stained carrier peptides, except in the case of coat protein peptides that lack valine. We conclude that this material is chemically indistinguishable from TMV coat protein.

We next asked whether the putative coat protein could be assembled into virus particles *in vitro*; that is whether it resembled coat protein by functional as well as chemical criteria. We therefore partially purified the *in vitro* synthesised coat protein by chromatography on DEAE-cellulose, a procedure which removes basic proteins which may bind nonspecifically to viral RNA during the assembly

process. After elution from the column, the protein was concentrated by precipitation at pH 5, dissolved in buffer (pH 7) and mixed with TMV RNA. After digestion with T1 RNase to remove uncoated RNA tails, the reconstitution mixtures were analysed by CsCl gradient centrifugation with the result shown in Fig. 3. Of four reaction mixtures, using the products labelled with either ^3H -leucine or ^{35}S -methionine, programmed by either coat protein mRNA or virion RNA, only the one with coat protein mRNA and ^3H -leucine as label gave radioactively labelled nucleoprotein of the same buoyant density as added $^{32}\text{PO}_4$ -labelled TMV. Furthermore, the labelled protein extracted from this reconstituted nucleoprotein migrated with authentic TMV coat protein on polyacrylamide gels, and no other labelled bands could be detected on the fluorogram. This was in marked contrast to the starting material, which contained many other labelled polypeptides besides coat protein. We conclude from these experiments that a small RNA species present in TMV-infected cells, and not an artefact of extraction, programmes the *in vitro* synthesis of a protein

which is chemically and functionally indistinguishable from TMV coat protein.

Characterisation of the RNA

Up to this point, the fractionation of RNA by sucrose density gradients provided only partially pure coat protein mRNA, but one would expect the coat mRNA activity to reside in a discrete RNA species if it were of genuine biological significance. We therefore took our most active fraction of gradient purified RNA and fractionated it further on an Agarose/acrylamide gel¹⁹. Figure 4 shows a gel stained with Stainsall²⁰; adjacent tracks contain RNA from TMV-infected and uninfected tobacco leaves. We found a reproducible minor stained band in infected leaves which was absent in control leaves. To see if it was coat protein mRNA, a gel with duplicate tracks was run, and one track was stained while the other was cut into bands and the RNA was extracted. We found that RNA from the new gel band was an excellent mRNA for coat protein (Fig. 4). Some of the slower migrating bands also programmed the synthesis of small amounts of coat protein, besides making larger proteins as one would expect. We do not know whether this represents a certain amount of trailing on the gel or of aggregation (since these were not denaturing gels), or whether there may perhaps be larger RNA molecules with coat protein mRNA activity. We estimate that the molecular weight of the major mRNA species is about 2.5×10^5 , or 750 nucleotides, of which at least 480 would be required to code for coat protein. Coat protein mRNA failed to bind to oligo(dT)-cellulose (data not shown), showing that, like TMV virion RNA, the coat protein message lacks a significant poly(A) region.

From the classical work mentioned in the introduction, we expected the coat protein mRNA to have the same polarity as virion RNA, and since large end products of RNase T1 digestion which come from the coat cistron have already been characterised^{4,21}, we sought these characteristic markers in coat protein mRNA. Tobacco plants were infected with TMV and labelled by growing them in medium containing $^{32}\text{PO}_4$. Coat protein mRNA was extracted as before, and purified on sucrose gradients and Agarose/acrylamide gels. We found the expected marker oligonucleotides in fingerprints of the T1 RNase digests (Fig. 5b), thus proving directly that the gel band contained nucleotide sequences from the expected region of virion RNA, so that it is a fragment of the viral (+) strand, and does not come from an unrelated RNA, such as a satellite virus. There was another unexpected bonus from this analysis, in that the fingerprint bore a striking resemblance to that of an RNA extracted from a nucleoprotein fragment of defined length (containing 1,000 nucleotides) which is generated when TMV particles are degraded in mildly alkaline conditions^{22,23}. The RNA from this 'alkali stable fragment' has independently been shown to contain the 3' terminal T1 oligonucleotide of TMV in molar yield²⁴, and can be charged with histidine by yeast activating enzymes, as is the case for intact TMV RNA²⁵. Analysis of the T1 RNase fingerprint of this fragment showed that it also contained the marker oligonucleotides found in the coat protein mRNA (Fig. 5a), showing that the part of the coat cistron from which they originate must be within 1,000 nucleotides of the 3' end of TMV RNA. Furthermore, the purified coat protein mRNA was found to contain a T1 oligonucleotide which gave rise to the pancreatic RNase products (AAU, C₅G). This is probably identical with the oligonucleotide AAUCCCCCG which occurs about 20 nucleotides from the 3' end of intact virion RNA²⁶. This suggests that the coat protein mRNA probably corresponds to the 3' terminal 750 nucleotides of virion RNA.

This assignment of the location of the coat protein cistron is at the opposite end of the RNA to that found by Kado

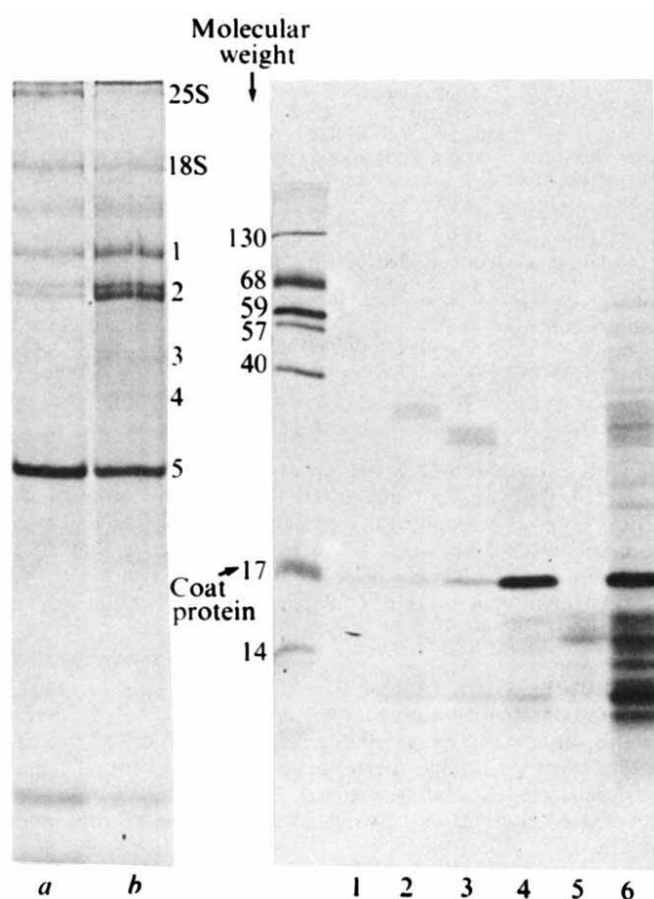


Fig. 4 Fractionation of the coat protein mRNA by gel electrophoresis. On the left are shown two tracks from a 2.8% Agarose/acrylamide gel stained with Stainsall (Eastman Chemicals). *a*, 6–18S RNA from uninfected tobacco leaves; *b*, 6–18S RNA from TMV-infected leaves. The indicated bands from parallel unstained tracks were cut out and the RNA was extracted from them by homogenisation in an equal mixture of phenol and buffer containing 0.1 M NaCl, 10 mM Tris-Cl, pH 7.5, 1 mM EDTA, 1% SDS, with 50 μg of carrier yeast tRNA. After extraction for 6 h at 0°C, the tubes were centrifuged and RNA precipitated from the supernatants with 2.5 volumes of ethanol. The precipitates were washed with ethanol before being taken up in water and tested for template activity in the wheat germ system as described in Fig. 1. The autoradiograph on the right shows the analysis on a 20% acrylamide gel of the products specified by fraction 14 RNA (track 6) and RNA from the indicated gel bands (tracks 1–5). The positions of molecular weight markers are indicated in the left margin of the gel.

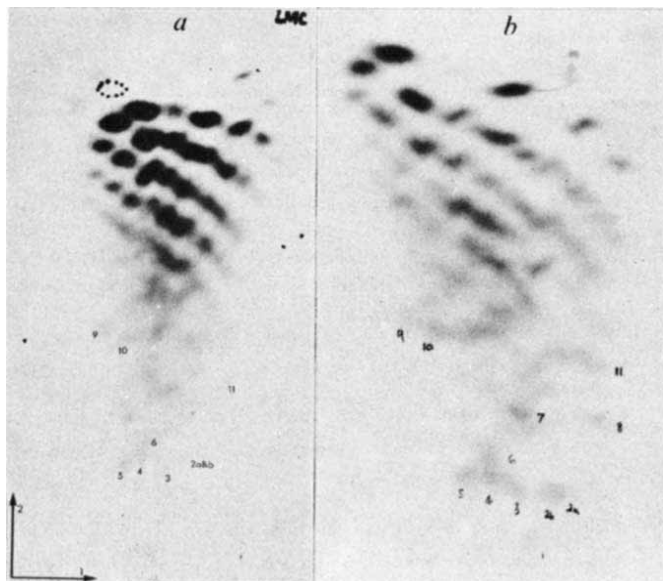


Fig. 5 Comparison of T1 RNase digested coat protein mRNA and 'stable fragment' RNA. ^{32}P -labelled TMV was prepared as described by Zimmer⁴⁰ and incubated at 4 °C overnight in 10 mM $\text{NaHCO}_3/\text{Na}_2\text{CO}_3$, pH 10.2. Uncoated RNA was digested with micrococcal nuclease ($1 \mu\text{g ml}^{-1}$) in the same buffer adjusted to pH 8.0 with HCl and 1 mM CaCl_2 . EDTA was added in excess to stop the digestion, and the RNA that had been protected from digestion extracted with phenol-SDS, and purified by sucrose density gradient centrifugation. Fractions containing 'stable fragment RNA' were pooled and precipitated with 2.5 volumes of ethanol. ('Stable fragment' from ^{32}P -labelled TMV was prepared by Michael Wilson.) The precipitate was taken up in 10 mM Tris-Cl, pH 7.6, 1 mM EDTA and digested with 3 μl of RNase T1 (1 mg ml^{-1}) in the presence of carrier *E. coli* tRNA (50 μg). The digest was fingerprinted as described by Barrell⁴⁴. Spot 2b is AUAAUAAUUUAUAGp, which codes for residues 125–129 of coat protein⁴. Spot 5 is AAACCUUCACCACAAGp, which codes for amino acids 53–57^{4,21}. Spot 9 is AACCCACGp, coding for residues 101–103⁴. Spot 4 can also code. Spot 10 here is a mixture, but contains a component giving pancreatic RNase products AAU, Cn, G, chain length 9–10, consistent with AAUCCCCCGp which comes from positions –20 to –28 from the 3' end of whole TMV RNA (ref. 26). The left hand autoradiograph shows the digest of band 4 extracted from a gel similar to that shown in Fig. 4, except that the RNA was prepared from leaves of plants infected with TMV grown in $^{32}\text{PO}_4$. Spots 2a–11 were identified by their pancreatic RNase digestion products as being identical with the equivalent spots in the fingerprint of stable fragment RNA. The more complex appearance of this fingerprint is due to slight pancreatic RNase digestion, which probably occurred during the lengthy extraction process; the mRNA was also slightly contaminated with ribosomal RNA fragments. *a*, Coat protein mRNA; *b*, 'Stable fragment RNA'.

which serve in turn as templates for the synthesis of progeny virion RNA molecules.

The synthesis of coat protein is delayed until its functional mRNA has been produced, because virion RNA does not serve as a template for coat protein synthesis. Two plausible mechanisms for production of the coat protein message are (1) that it is released from virion RNA by specific cleavage, and (2) that it is synthesised by a replicase which initiates transcription at a specific internal site on the complementary strand of TMV RNA. We cannot distinguish between these possibilities at present.

Only when enough coat protein has accumulated to permit the formation of 'disk' aggregates³¹ can the assembly of virus particles begin³². This would delay the encapsidation of virion RNA thereby allowing time for synthesis of early proteins and (–) strands. Once assembly begins the virion RNA will be sequestered and therefore can no longer be a template for either process. But since neither (–) strands nor coat protein mRNA are encapsidated in the case of TMV vulgare, the production of virions can continue for as long as these RNA molecules remain intact, and the replicase(s) active.

This kind of scheme can easily explain how TMV can produce such large quantities of coat protein compared with its other proteins. Being a helical virus, TMV requires many more protein subunits to encapsidate its genome than would a spherical virus with the same genome size³³. Thus poliovirus, whose RNA is slightly larger than that of TMV, requires only 60 copies³⁴ of each structural polypeptide compared with about 2,100 in the case of TMV.

Translational control

We do not know why the coat protein cistron is untranslatable in intact TMV RNA whereas it is easily read in the coat protein mRNA; however, at least two precedents offer clues. Initiation of translation of the A cistron of the RNA phages seems to occur only in short growing RNA molecules, since the initiator region of this cistron is involved

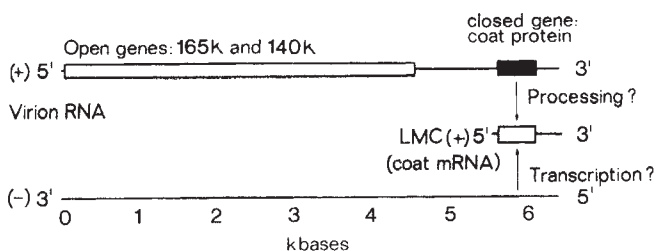


Fig. 6 A tentative map of TMV RNA and its known proteins. The map is drawn to scale. The location of the coat cistron is discussed in the text; the location of the 165,000 and 140,000 molecular weight polypeptides is less certain. Since the sum of their molecular weights exceeds the coding capacity of the virion RNA, they must overlap somehow. We believe they are initiated at different sites since virion RNA can bind two ribosomes simultaneously and independently in the presence of 10^{-4} M sparsomycin, promoting the formation of two methionyl dipeptides: Met-Ala and (probably) Met-Thr (T.R.H. and T.H., unpublished observations). The simplest possibility is that they then terminate at the same site, although this need not necessarily be the case. Their position near the 5' end of the RNA is suggested by the fact that RNA molecules with small deletions at the 5' end, constructed by alkali stripping procedures similar to those described in the legend to Fig. 5, fail to synthesise either large polypeptide (T.R.H., M. Wilson, and D.Z., unpublished results). This again is inconsistent with the possibility that the coat protein gene is contained within the large polypeptides (see above). These assignments leave approximately 1,100 residues of RNA unaccounted for, enough to code for up to 40,000 daltons of protein, and this gap has been positioned in the middle of the map. There is evidence from another strain of TMV (the cowpea strain) that a 28,000–30,000 molecular weight protein, whose translation is regulated by a mechanism similar to that described here for TMV coat protein, may be coded in this region⁴⁹.

and Knight²⁷ on the basis of SDS stripping and marker rescue *in vivo*, but agrees with that of Richards *et al.*²⁸.

Virus replication cycle

On the basis of this evidence, we propose the following account of the replicative cycle of TMV (Fig. 6). After infection of the cell and uncoating of the virion RNA, ribosomes can only translate the cistrons for the 140,000 and 165,000 molecular weight polypeptides, consistent with their early appearance in infected protoplasts. We assume that these functions are required early in infection, and since the subunit molecular weights are close to the apparent native molecular weight of TMV replicase activity in partially purified extracts^{29,30}, we suggest that both proteins are viral replicases, or their subunits; the function of each being different. For example, one of them may use the (+) strand as a template, the other the (–) strand. Alternatively, one may make coat protein mRNA by partial transcription of the (–) strand, while the other makes full-length transcripts. At least one of them must catalyse the production of complementary copies of the virion RNA,

in stable secondary structure in the full-sized RNA molecule³⁵. Similar mechanisms may operate in brome mosaic virus (BMV), a multicomponent virus which contains four different RNA molecules. BMV RNA 3 is larger than BMV RNA 4, and just as TMV coat protein mRNA is wholly contained within virion RNA, so BMV RNA 4 is contained within BMV RNA 3 (ref. 36); furthermore, BMV RNA 4 codes efficiently for BMV coat protein, whereas BMV RNA 3 makes another polypeptide but little coat¹⁷. It seems probable that the coat cistron in BMV RNA 3 is masked by secondary structure, but there is another possibility which may either augment or replace this mechanism. When the sequence of the ribosome binding site of BMV RNA 4 was determined³⁸, the 5' end was found to be blocked with m⁷G; this unusual end group may be necessary for ribosomes to attach to mRNA³⁹. We do not know whether TMV coat protein mRNA has this structure at its 5' end or not, though intact virion RNA certainly does⁴⁰.

Such regulatory mechanisms are not confined to plant and bacterial RNA viruses, however; in both Sindbis and Semliki Forest virus-infected cells small RNAs with the same polarity as the packaged RNA have been found which, unlike their parent molecules, efficiently direct synthesis of the corresponding coat polypeptides *in vitro*^{41,42}. Even in the cases of the DNA viruses polyoma and SV40 it seems that larger RNA molecules which code for one protein code for a different protein after cytoplasmic cleavage⁴³. Whether such a mechanism regulates the translation of normal cellular messages is unknown. It might account for some cases of 'masked mRNA', for example. We would like to conclude by pointing out that these cases of translational control operate with high efficiency, yet they do not appear to require the action of specific protein synthesis initiation factors.

A preliminary account of these experiments has been published⁴⁸.

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letters to nature

Clusters of galaxies as gravitational lenses?

KAROJI and Nottale¹ have compared galaxies situated behind or inside clusters with those whose light does not encounter any important cluster of galaxies. They suggested that either light emitted by distant galaxies is redshifted when passing through clusters of galaxies, or distant sources appear more luminous when seen through intermediate clusters of galaxies, which could act as gravitational lenses. Here we examine the magnitude of the lens effect for clusters of galaxies, and find it unlikely that the observational effect claimed by Karoji and Nottale can be explained in this way.

Consider a source of radiation at a distance $L \simeq cz/H_0$ from the observer, in a universe which is homogeneous on some sufficiently large scale, with mean density ρ_u . The line of sight to the source may, however, be such that the mean density along the beam of light, $\langle \rho \rangle$, differs from ρ_u . The cross sec-

tional area, A , of such a beam of light is governed by the equation^{2,3}

$$(A')'' + 3\alpha\sigma_0(1+z)^6 A^{\frac{1}{2}} = 0 \quad (1)$$

where $\alpha \equiv \langle \rho \rangle / \rho_u$ is the density enhancement along the beam, $\sigma_0 \equiv 4\pi G \rho_u / 3H_0^2$ is the cosmological density parameter, and ' denotes differentiation with respect to an affine parameter along the beam of light. The solution of equation (1) for such a 'zero-shear' beam shows that a distant source which would have an apparent magnitude m in a strictly homogeneous universe has an apparent magnitude $m + \Delta m$ when seen along this line of sight, where

$$\Delta m = 1.086\sigma_0(1-\alpha)z^2\{1+O(z)\} \quad (2)$$

It follows that for $\alpha < 1$, there will be a dimming of distant

sources³. When looking through a cluster of galaxies, however, $\alpha > 1$ and we expect background objects to be intensified.

To estimate the parameter α when the line of sight passes through a cluster, we consider a cylinder of small diameter along the line of sight, and calculate the mean density, $\langle \rho \rangle$, within that cylinder. Since we are interested in clusters of galaxies, we shall use the approximate density law found by King⁴ for the Coma cluster

$$\rho(r) = \rho_c \{1 + (r/a)^2\}^{-3/2} \quad (3)$$

where ρ_c is the central density, r is the radial coordinate for the spherically symmetric cluster, and a is the distance at which the density has fallen off to $2^{-3/2}\rho_c$. We assume that the cluster has a radius R , defined to be the distance at which the cluster density fades into the universal background density, that is

$$\rho_c/\rho_u = (1 + (R/a)^2)^{3/2} \quad (4)$$

For a line of sight with impact parameter Rh , $0 \leq h \leq 1$, the density enhancement is then found to be

$$\alpha \simeq 1 + 2 \frac{a\rho_c}{L\rho_u} (1 - h^2)^{3/2} \left\{ 1 + \left(\frac{h}{u} \right)^2 \right\} \quad (5)$$

where $u = a/R \simeq (\rho_u/\rho_c)^{1/3}$.

The intensification, in magnitudes, is then given by

$$\Delta m(h) \simeq -2.172 (\sigma_c) \frac{a}{c/H_0} z (1 - h^2)^{3/2} \left\{ 1 + \left(\frac{h}{u} \right)^2 \right\} \quad (6)$$

where $\sigma_c \equiv 4\pi G\rho_c/3H_0^2$ is the density parameter at the centre of the cluster. It is also of interest to obtain the average intensification, $\langle \Delta m \rangle$, over the area of the cluster

$$\frac{\langle \Delta m \rangle}{\Delta m(0)} = u^2 \{ (1 + u^2)^{3/2} \ln \left(\frac{(1 + u^2)^{1/2} + 1}{(1 + u^2)^{1/2} - 1} \right) - 2u^2 - \frac{8}{3} \} \quad (7)$$

For this approach to be valid, the focal length, $f(h)$, of the gravitational lens must be significantly larger than the source- and observer-lens distances, and it must be assumed that the cluster lies roughly halfway between source and observer. The last restriction is not very stringent, for roughly halfway means really that neither the source nor observer lies right next to the cluster when compared to the other.

The angle of deflection for photons passing the lens at a distance Rh from its centre is given by

$$\theta(h) = 4GM_c(h)/c^2 Rh \quad (8)$$

where $M_c(h)$ is the mass contained within the cylinder, of radius Rh , whose axis passes through the centre of the lens, parallel to the line of sight⁵. By considering radially adjacent lines of sight with impact parameters Rh and $R(h+dh)$, the focal length is found to be

$$f(h) = R \left(\frac{d\theta}{dh} \right)^{-1} = \frac{c^2 R^2}{4G} \left\{ 2 \frac{dM_c}{d(h^2)} - \frac{M_c}{h^2} \right\}^{-1} \quad (9)$$

For the King density distribution, we find

$$M_c(h) = 4\pi a^3 \rho_c \left\{ \ln \left(\frac{(1 + (1 + u^2)^{1/2})(1 + (h/u)^2)^{1/2}}{(1 - h^2)^{1/2} + (1 + u^2)^{1/2}} \right) - \frac{1 - (1 - h^2)^{1/2}}{(1 + u^2)^{1/2}} \right\} \quad (10)$$

which gives for the focal length of such a lens

$$\frac{f(h)}{f(0)} = \left(\frac{1}{2} \right) \left(\frac{h}{u} \right)^2 \left\{ 1 - \frac{(1 - h^2)^{1/2}}{1 + (h/u)^2} - (1 + u^2)^{1/2} \times \right. \\ \left. \times \ln \left(\frac{(1 + (1 + u^2)^{1/2})(1 + (h/u)^2)^{1/2}}{(1 - h^2)^{1/2} + (1 + u^2)^{1/2}} \right) \right\}^{-1} \quad (11)$$

where

$$f(0) = \frac{(c/H_0)^2}{6\sigma_c a} (1 + u^2)^{1/2}$$

is the focal length through the centre of the lens.

If we adopt the parameters given by King for the Coma cluster (noting that he used $H_0 = 75 \text{ km s}^{-1} \text{ Mpc}^{-1}$), we then find $\sigma_c = 2.1 \times 10^4$. It follows that for this cluster $f(0) \simeq 750 \text{ Mpc}$ and $\Delta m(0) = -1.94z$, where z is the redshift of the source observed through the cluster. Since the mean radial velocity of the objects found by KN to lie behind clusters is $11,600 \text{ km s}^{-1}$, the average intensification of such an object observed through the centre of the Coma cluster should be $\Delta m(0) \simeq -0.076 \text{ mag}$. Objects not observed through the centre of the cluster will, of course, be less intensified, as indicated by equation (6). If the object being observed lies inside the cluster, there will be a range of α depending upon the position of the object, and hence a greater range of Δm . In fact, a proper treatment of this case would require a detailed knowledge of the parameters of the cluster and the position of the source within it, but we think it unlikely that the maximum intensification would be more than a factor of two different from $\Delta m(0)$ above.

Our value of $\Delta m(0)$ is considerably less than the mean magnitude differences (-0.26 ± 0.218 and -0.43 ± 0.237) found by Karoji and Nottale, although their error limits are large. It must also be remembered that many of their objects are being seen through clusters which are considerably smaller, and presumably less condensed centrally, than Coma. This suggests that, if the large magnitude differences found by Karoji and Nottale are real, then 'gravitational lensing' is unlikely to be the cause.

Because galaxy magnitudes are notoriously difficult to measure precisely, we suggest, however, that it may be worthwhile to make an accurate, systematic investigation of the apparent magnitudes of a homogeneous, distant sample of galaxies seen through, and outside of, a cluster of galaxies such as the Coma cluster. The general theory of relativity predicts that the lensing effect must be present; our calculation indicates that measurements with an accuracy of $\sim 0.01 \text{ mag}$ will be necessary to find it. This is clearly at the limit of accuracy obtainable in measurements of galaxy magnitudes⁶. If the effect were found, however, it would provide not only further verification of general relativity, but also a means of probing the mass of a cluster of galaxies, independent of assumptions about the mass to luminosity ratio of its constituents.

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Decametric radio emission from four pulsars

WE have reported earlier¹ on the reception at low radio frequencies of radio pulses from three pulsars. A similar method² was used during 1973–74, and four more pulsars were detected.

The signals were fed into 16 receivers with analog stores giving an equivalent time constant of 100 s. The receiver central frequencies and bandpass values were chosen to allow for frequency dispersion, giving a time resolution of $T/16$, where T is the period of the pulsar. The source was tracked for periods of 1–5 h during the measurement. Stored signals were recorded at intervals of 180–200 s.

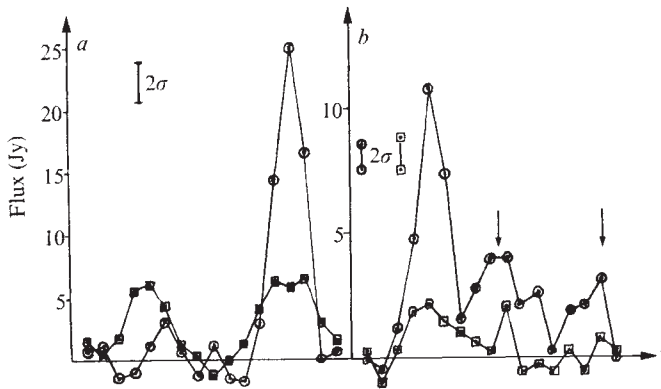


Fig. 1 Integrated pulse profiles for PSR0950+08. *a*, For 1973 March 27, taken over 2,400 pulses. *b*, For 1973 March 1, taken over 23,000 pulses at 25 MHz and 16,000 pulses at 20 MHz. $\odot$, 25 MHz data, at 80 kHz bandwidth; $\square$, 20 MHz data at 40 kHz bandwidth.

The signals were averaged over time as well as frequency channels. The signals from the separated frequency bands were superposed with a time delay calculated from known values of the dispersion measure.

At low radio frequencies the integrated pulse profiles are wider than at high frequencies, and they contain new components (interpulses). We distinguished these components from noise by

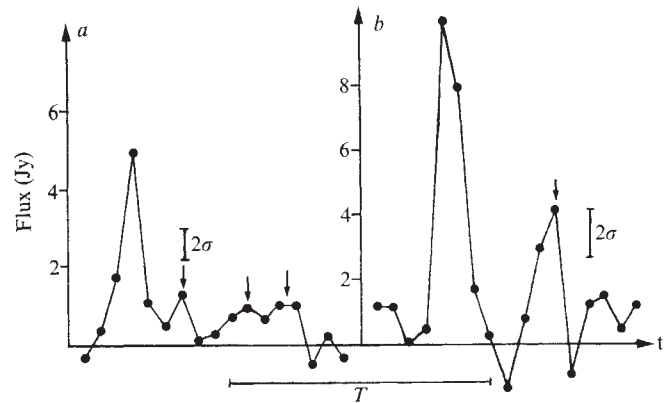


Fig. 2 Integrated 25 MHz pulse profiles for PSR0834+06. *a*, 3,000 pulses on 1973 March 7 and 1973 March 27 at 160 kHz bandwidth. *b*, 1,700 pulses on 1973 April 17, at 80 kHz bandwidth.

setting a threshold level which depended on the r.m.s. noise level, taking account of the width of the components and the error in determining the mean flux³. Some observational results are presented in Table 1, together with the main parameters of the equipment.

PSR0950. As at higher frequencies this pulsar shows a high sporadicity. Shown in Fig. 1 are two typical repeated forms of the integrated pulse profile, recorded at 25 MHz and at 20 MHz. In this and the following figure the 2σ noise level is shown. The main pulse is followed either by one interpulse at $0.4T$ (Fig. 1*a*) or by two at $0.3T$ and $0.7T$ (Fig. 1*b*), which overlap and cover the entire period. This behaviour may be compared with the interpulse observed at high frequencies^{4,5}, which occurs $0.6T$ after the main pulse.

PSR0834. This pulsar has a lower intensity and it was detected only at 25 MHz. Its integrated profile is very complex (Fig. 2). For 50 per cent of cases it showed an interpulse $0.4T$ after the main pulse; two more interpulses appear rather seldom. The points of minimum emission are stable.

PSR1237. Both this pulsar, and PSR2016, are characterised by a high sporadicity and low intensity. Figure 3 shows two typical

Fig. 3 Integrated 25 MHz pulse profiles for PSR1237+25. *a*, Taken from 1,200 pulses on 1973 January 17: — 110 kHz bandwidth, --- 110 kHz bandwidth, — 220 kHz bandwidth. *b*, 800 pulses on 1973 February 7: — 110 kHz, --- 110 kHz, — 220 kHz bandwidths. *c*, 1973 April 4: — 110 kHz; 1973 April 10: --- 110 kHz bandwidth; combined data: —, 500 pulses. *d*, 1973 March 29: 1,800 pulses; — 110 kHz, --- 110 kHz, — 220 kHz bandwidths.

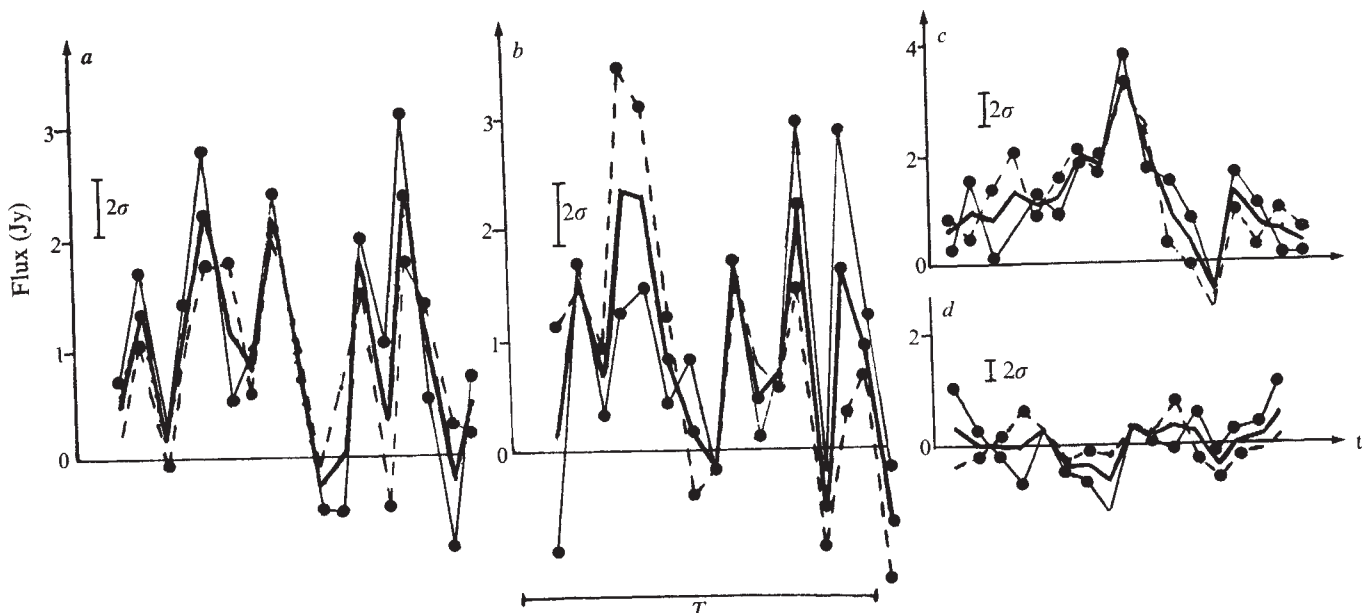


Table 1 Observational results

PSR	Frequency range (MHz)	Band of 1st channel (kHz)	Detuning between the adjacent channel (kHz)	Average flux $\times 10^{26}$ ($\text{W m}^{-2} \text{Hz}^{-1}$)	No. of interulses	Width of the main pulse (ms)
0834	25	10	12.00	0.94	0-4	130
0950	25	8-10	9.94	1.7	0-2	35
0950	20	4-5	5.19	0.75	1-2	65
1237	25	14	17.19	1.0	0-4	—
2016	25	4	4.63	0.2	1	35

integrated profiles obtained at different times. Some features are common to Fig. 3a and b, and to Fig. 3b and c, but there are obviously very large differences. Figure 3d shows a corresponding record when the pulsar signal was not observed.

PSR2016. The integrated profile is shown in Fig. 4. Again the profile is complex, showing a main pulse and an interpulse.

An important conclusion can be drawn from these results and from those of our earlier investigation¹. The integrated pulse profile, or 'emission window', for all of the seven pulsars so far investigated, covers almost the entire period and contains

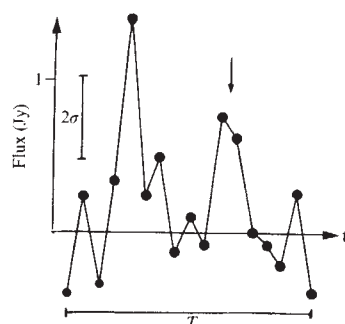


Fig. 4 Integrated 25 MHz pulse profile for PSR2016+28. 10,000 pulses taken on 1972 October 3 at 64 kHz bandwidths.

intense interulses. This effect seems to be a typical behaviour of the decametric radio band.

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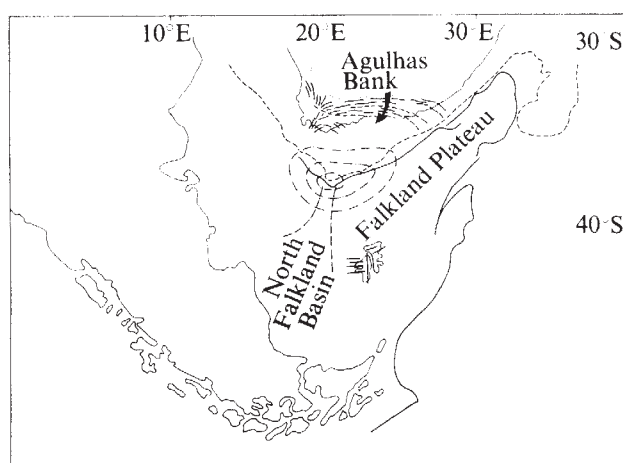
⁵ Lyne, A. G., Smith, P. G., and Graham, D. A., *Mon. Not. R. astr. Soc.*, **153**, 337 (1971).

there became a combination of spreading and transform motion (ref. 1, Fig. 1). An analogous set of movements was proposed for the Red Sea, Dead Sea, Gulf of Suez junction. Later, a large number of other triple junctions was recognised⁴, including a Georgetown RRR junction to the west which helped to define the course of the Africa/South America split and left the Takutu rift in Guyana as a failed arm filled with Cretaceous deposits.

So far, little attention has been paid to the southern end of the Atlantic spreading ridge in the reconstructions of the breakup of Gondwanaland, although the presence of a major transform (the Agulhas/Falkland Fracture Zone) is now well established. Little is known in detail about the Falkland Plateau, but the presence of the Falkland Islands, and the granitic basement found by deep drilling⁵ strongly suggest that the whole plateau consists of continental crust. Assuming this to be the case, and taking the 2,000-m isobath as defining the plateau edge, it is possible, using a slightly modified Bullard rotation, to fit the Falkland Plateau into the embayment of the distal Natal Valley, between the Agulhas Fracture and the southern Mozambique Ridge, with very little gap or overlap (Fig. 1). The South Atlantic spreading ridge and the Agulhas Fracture meet at approximately a right angle, and as Burke and Dewey⁴ have suggested that such angular bends commonly mark the site of triple junctions, it is worth enquiring whether a third arm exists. There is, in fact, evidence of a trough almost bisecting the angle made by the two other fractures; this is the North Falkland Basin, the present-day trend of which is north-east, and which lies north-west of the Falkland Islands. This contains⁶ up to 2 km of sediments, mainly of suggested late Cretaceous age.

Thus it is possible to postulate a triple junction consisting of the Atlantic spreading ridge, the Agulhas Fracture, and the North Falkland Basin—that is, a spreading ridge, a transform fault, and a failed arm—and it is proposed that this be called the Agulhas Triple Junction

Fig. 1 Proposed Agulhas Triple Junction, showing schematic contours of uplift (---) and relationship to the Cape Fold Belt.



Was there an Agulhas triple junction?

DISCUSSIONS on the opening of the South Atlantic in terms of major plate tectonic structures such as spreading ridges, transform faults, and so on, have tended to concentrate on the West African segments¹⁻³. Evidence has been presented for regarding the original break in the Gulf of Guinea as an RRR junction, the southward-trending arm becoming the Mid-Atlantic Ridge, the north-east-trending arm (Benue Trough) undergoing a relatively minor phase of opening followed by closing, and the westward arm becoming modified by a series of oblique shears so that movement

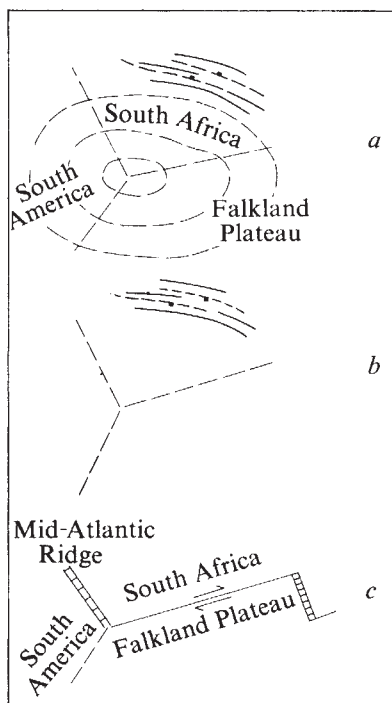


Fig. 2 Schematic representation of the proposed evolution of the Agulhas Triple Junction. *a*, Uplift and doming, radial and concentric fractures, formation of the Cape Fold Belt (early Triassic); *b*, subsidence with reversal of throw on Cape faults, no spreading, (?) initiation of Mesozoic basins (Triassic to Jurassic); *c*, initiation of separation, further faulting in Cape Fold Belt (early Cretaceous).

(Fig. 1). The similarity to the examples of the Gulf of Guinea, Georgetown and the Gulf of Suez is clear.

The idea of a possible Agulhas triple junction arose from studies in the Cape Fold Belt⁷, which resulted in the proposal of a gravity sliding model for the development of that orogen. This required that uplift should take place south of the present Fold Belt to form the slope on which sliding was initiated, and it was found that uplift centred on the proposed junction was well situated to do that. Since the initiation of triple junctions seems to depend on the strain developed over an uplift, probably resulting from a rising mantle plume⁴, it seemed natural to link the two concepts (Fig. 2). Upwelling must have been followed by subsidence (since no spreading occurred at this stage), which led to major southward downthrow on the large longitudinal faults associated with the Cape Fold Belt, and possibly also to the initiation of the Mesozoic basins of the South African continental shelf and coastal areas, which contain sediments as old as Triassic⁸. Separation only occurred later (125–130 Myr)⁹ but could have been controlled by the directions of the earlier-formed fractures.

I thank Professor R. V. Dingle for drawing my attention to the existence of the North Falkland Basin, and to C. J. Hartnady for the Bullard reconstruction.

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Shortening of continental crust in orogenic belts and plate tectonics

THE geotectonic and palaeogeographic problems created by severe crustal shortening in orogenic belts are numerous and difficult to solve. In spite of plate tectonics, or because of it, one such problem of major significance persists: where is much of the continental crust that once underlay many of the nappes in tremendously shortened and imbricated belts such as the Alps^{1–4}? In the scheme of plate tectonics, it is implausible to pull, melt or shove continental crust back into the mantle⁵; yet some authors express faith in such a concept⁶ or, lacking faith, despair of a solution⁷. I propose that the solution to this problems lies quite simply in the fact that the original eugeosynclinal basement⁸ of the Alpine belt consisted of distended continental crust, as clearly understood by Trümpy⁹.

It is possible to calculate the crustal shortening in the Eastern Alps by assuming constant crustal volume through time. The present crustal volume may be measured from a crustal model of the Eastern Alps based on detailed gravity and seismic studies¹⁰. In agreement with Dewey *et al.*¹¹, I assume that the volume of continental crust measured in the Alpine geophysical profiles is a reasonable approximation of the original volume of continental crust that existed before development of the Tethys and the Pennine Trough as well as before Alpine deformation. Crustal additions and subtractions from and by the mantle, the oceanic crust, and erosion and sedimentation (and also by volume changes) are thus considered relatively insignificant or mutually cancelling for the purposes of my calculations. The assumption of no crustal addition may not be far fetched, at least for the eastern Alps, considering the paucity of Mesozoic–Cainozoic magmatism and ophiolites in the area. The assumption of no crustal subtraction by erosion is clearly wrong for the Alps, but the evaluation of erosional losses would only amplify the conclusions of this study, and thus erosion is ignored. The assumption of no crustal subtraction by ‘oceanisation’ or disappearing continental blocks is reasonable, and is the principal point shown here.

Figure 1 shows the total measured crustal volume of a 1-km-thick slice of the 222-km-long profile to be 9.640 km³ with an average crustal thickness of 43.4 km. (For this calculation, made with a polar planimeter, I have subtracted out the chemical contribution of the volume of the Mesozoic carbonate rocks in the Northern Limestone Alps.) Assuming an initial normal crustal thickness of 30 km, it follows that an initial length of section of 321 km would be required to maintain constant volume. This would require a shortening of nearly 100 km in the present state. This number does not compare with geological estimates of 180–600 km of shortening^{1,3,4} of the palaeogeographic realms underlain by continental crust in the Eastern Alps.

The Red Sea region can be used as a valid geotectonic model of the Triassic–Jurassic Alpine belt, representing an initial crustal configuration in which palaeogeographic realms are sited on distended continental crust (see refs 12 and 13). From the profile (Fig. 2), a simple analysis as described in Fig. 3 results in the values shown in Table 1.

Table 1 Parameters from Red Sea and Alpine regions

Length of Red Sea profile	449 km
Shorten profile to subtract all oceanic crust	– 82 km
Shorten to restore continental crust to original thickness (30 km thick)	– 146 km
Shorten to thicken continental crust to 43.4 km average of present Eastern Alps	– 69 km
Final width of the profile	152 km

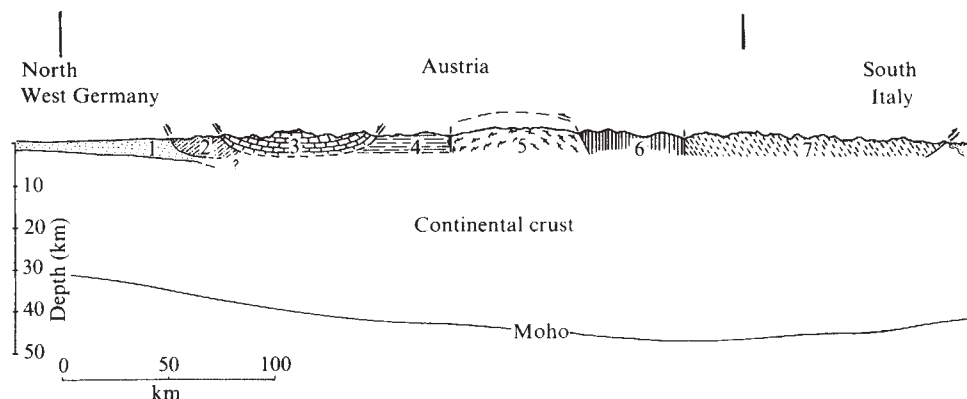


Fig. 1 Crustal profile through the eastern Alps from near Chiemsee, West Germany (north), in the molasse zone, to Maniago, Italy, in the Po Basin (adapted from Angenheister *et al.*¹⁰). 1, Molasse; 2, flysch and Helvetic zone; 3, Northern Limestone Alps; 4, greywacke zone; 5, Tauern fenster; 6, Altkristallin; 7, Southern Alps.

Assuming the effective removal of the oceanic crust without trace, the shortening of the continental crust is 215 km.

In order to attain the final dimensions of the Alpine profile for direct comparison, I have added 70 km of length to reach 222 km (Fig. 1). Since this 70 km must have a final thickness of 43.4 km, it is actually necessary to add 100 km of crust 30 km thick to the initial profile (Fig. 2). That is, the Alpine crustal profile balances in volume with a Red Sea profile that includes an extra 100-km-long stretch

may, therefore, be eliminated on consideration of the simple fact that initial thinning of continental crust of orogenic belts has not until now been taken into account in models of crustal shortening.

Of course, I am aware that many factors contribute to changes of crustal volume and that the deformation of colliding continental blocks, in particular, must be complex in space and time¹⁴⁻¹⁶. This paper serves only to emphasise the critical importance of initial crustal configuration when

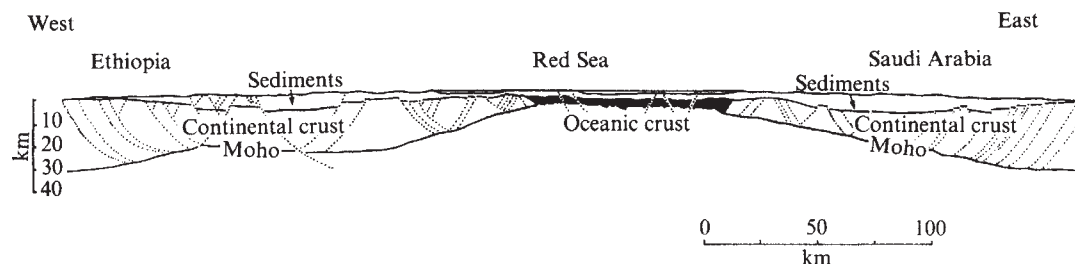


Fig. 2 Crustal profile through the southern Red Sea from Ethiopia (west) to Saudi Arabia, based upon gravity, seismic, magnetic and bore hole data (adapted from Lowell and Genik¹²). The extreme thinning of continental crust shown in this profile is not agreed upon by all workers, but is considered to model the Alpine region in the Triassic-Jurassic. This profile is not entirely perpendicular to regional strike. In calculations, the sediments are not included in the volume of continental crust in as much as they are largely chemical precipitates.

of normal continental crust beyond that shown in Fig. 2. Therefore, the final dimensions of the crustal shortening model developed are as shown in Table 2.

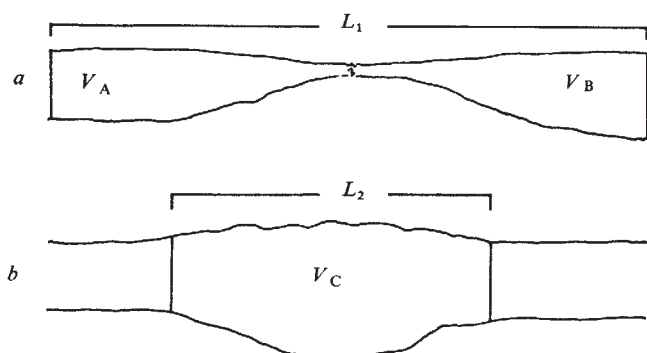
Table 2 Dimensions in the crustal shortening model	
State	Dimensions of profile
Initial stage of continental distension (Red Sea model)	549 km long (82 km oceanic length) 9,640 km ³ per km continental crust Continental crust varies from 0 to 30 km thickness
Final stage of orogenic shortening (Eastern Alps)	222 km long 9,640 km ³ per km continental crust Continental crust 43.4 km average thickness 245 km of crustal shortening on continental basement

This model of crustal shortening, though based on gross and simplifying assumptions, seems to provide an explanatory step in the right direction. For, along with a geologically reasonable estimate of crustal shortening over continental basement (245 km), it also eliminates the need for disappearance of large volumes of continental crust, a concept distasteful to fundamental physical principles. The problem of excessive crustal shortening in orogenic belts

considering problems of orogenic crustal shortening. The application of this result to the study of orogenic tectonic evolution will be of interest.

I am grateful to Professor A. Tollmann for his assistance and for providing a Gastdozent position at the University

Fig. 3 Method of calculation. The crustal volume in profile of two rifted continental margins (a) is set equal to the crustal volume of the orogenic profile (b). Knowing the length L_2 , L_1 can be calculated to satisfy the balance $V_A + V_B = V_C$. The amount of shortening on continental basement is $L_1 - L_2$. The horizontal dimension of any oceanic crust in profile (a) is eliminated because it and its overlying sediments (except chemical sediments that must be subtracted) cannot enter into the crustal balance as discussed in the text.



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Biostratigraphy of some marine manganese nodules

USING scanning electron microscopy (SEM) we have examined the microtexture of Mn nodules. Previous researches using SEM observation on marine Mn nodules have revealed many characteristic features which may relate to their formation^{1–6}.

We report here the discovery of *in situ* calcareous nannoplankton fossils in a Mn nodule collected during the RV Hakuho-Maru Cruise KH-74-4, and present a preliminary calculation of the growth rate, using a biostratigraphic method. We also discuss the potential value of Mn nodule biostratigraphy⁷ in terms of deep sea palaeoenvironments.

The Mn nodules were dredged from a brown clay by a Norwalk type dredge at the summit of Makarov Guyot (29°26.3'N, 153°27.3'E; water depth, 1,378 m)⁸. One of them (16002) is

spheroid in shape, with concentric symmetric layers around a basaltic rock nucleus (Fig. 1a). The crust, about 60 mm thick, comprised three layers: an outermost, 2 mm thick with a bumpy surface texture (Fig. 1b); a middle, about 8 mm thick, comprising many thin lamellae; and the innermost, about 50 mm thick, massive and dense in texture.

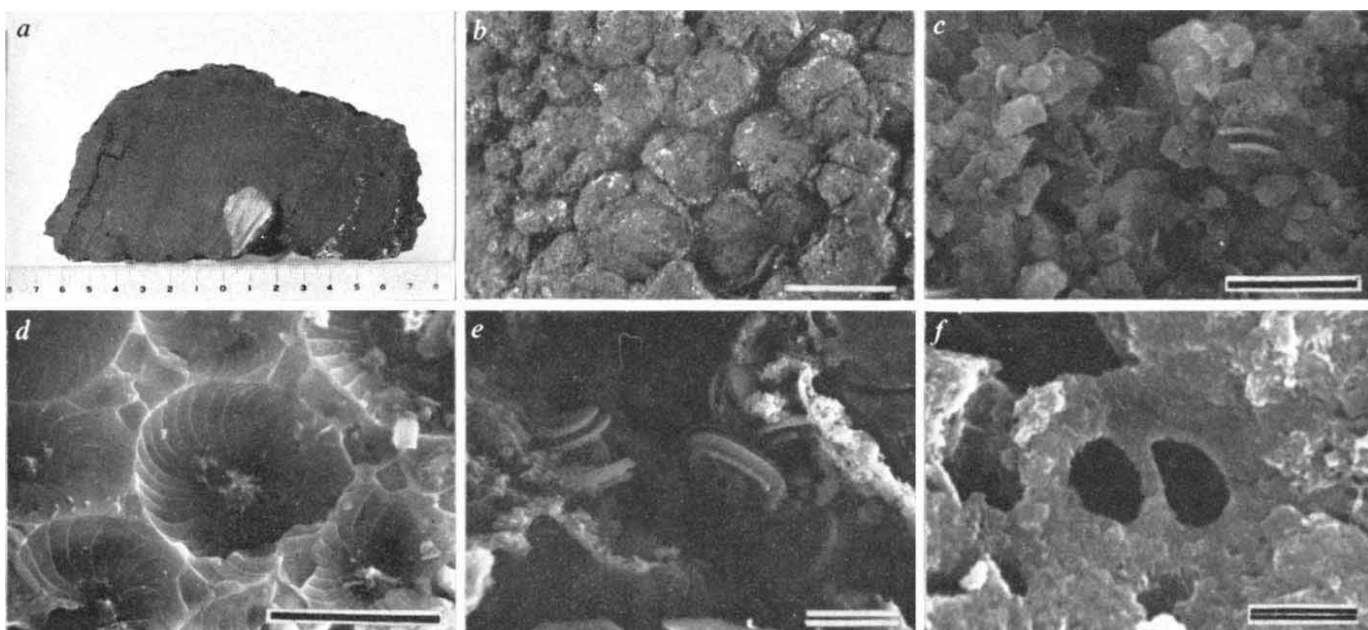
The outermost layer and the innermost layer adjacent to the nucleus were peeled off by hand after drying at room temperature, and were coated with carbon and gold thin films for SEM observation.

The ooze adhering to the surface of the outermost layer comprised calcareous nannoplankton fossils: *Coccolithus pelagicus* (Wallich) Schiller; *Cyclococcolithina leptopora* (Murray and Blackman) Kamptner; *Emiliania huxleyi* (Lohmann) Hay and Mohler; *Gephyrocapsa oceanica* Kamptner; and *Umbilicosphaera mirabilis* Lohmann. The assemblage represents NN21 zone (0.2 Myr–present)⁹.

The surface of the outermost layer itself is also covered with calcareous nannoplankton fossils. The microtexture and the presence of *E. huxleyi* (Fig. 1c) indicate the recent growth of the nodule.

The inner surface of the outermost layer also contains calcareous nannoplankton fossils preserved as impressions (Fig. 1d) or replaced by ferromanganese oxides (Fig. 1f). Although most are too poorly preserved, several species have been identified: *C. pelagicus*; *Cyclococcolithina macintyreii* Bukry and Bramlette; *Cyclococcolithina leptopora*; *Pseudomilania lacunosa* (Kamptner) Gartner; and *U. mirabilis*. The assemblage indicated NN19 zone (2–0.5 Myr). The presence of a coccosphere of *Cyclococcolithina leptopora* (Fig. 1d) suggests that the fragile coccosphere itself may have been covered with ferromanganese oxide soon after the deposition on to the nodule surface, which subsequently dissolved away after consolidation of the oxide. Likewise, the other microfossils are considered to have been rapidly incorporated into the layer, probably simultaneously with the ferromanganese oxide. Besides the late Neogene calcareous nannoplankton fossils, several individuals of *Chiasmolithus grandis* Hay, Mohler and Wade (Fig. 1e), an Eocene index species, were found inside a cavity in the outermost layer. Since, unlike the other *in situ* microfossils, they were observed only in the cavity,

Fig. 1 Photographs (a, b) and SEM photomicrographs (c–f) of a marine Mn nodule. a, Tangential section showing three major layers: thin outer layer; middle layer with many thin lamellae; and massive innermost layer (scale, 1 cm). b, Detail of the surface texture of the outermost layer. Bumps were peeled off for SEM observation (scale, 1 cm). c, Enlarged surface texture of the outermost layer after ultrasonification. *E. huxleyi* embedded in the layer indicates the recent growth of the nodule (scale, 5 µm). d, Impression of a coccosphere of *Cyclococcolithina leptopora* suggesting that most of the microfossils were incorporated into the layer rapidly (scale, 5 µm). e, *Chiasmolithus grandis* (scale, 20 µm). f, *G. oceanica*, replaced by Mn oxides, in the innermost layer adjacent to the nucleus (scale, 1 µm).



apparently unaltered, they may have resulted from the reworking of the sediments.

In the innermost layer adjacent to the nucleus, *G. oceanica* (Fig. 1f), which first appeared at the beginning of the Pleistocene, were observed. No specimens of *Discoaster*, a species which became extinct at the end of the Pliocene, were found. Greenslate has suggested an important role for siliceous microfossils in nodule formation⁴; however, we have not recorded significant amounts of siliceous fossils (see refs 5 and 6).

Since the calcareous nannoplankton fossils preserved in the layers record the age of each layer, we can calculate the mean growth rate of each layer; other methods¹⁰⁻¹⁴ only give that of the outer layer.

The assemblages of the outermost layer indicate that it started to grow 2–0.5 Myr ago, and that it continued at least until 0.2 Myr ago. The mean growth rate of the outermost layer is thus 1.0–6.7 mm Myr⁻¹. The values calculated are compatible with those obtained by conventional methods: 1–100 mm Myr⁻¹ (ref. 15).

On the other hand, the presence of *G. oceanica* and the absence of the members of *Discoaster* in the innermost layer adjacent to the nucleus suggest that the nodule is younger than 2 Myr old. Therefore, the minimum mean growth rate of the nodule, except for the outermost layer, is 39 mm Myr⁻¹. That seems reasonable, though it is about 5–40 times higher than values obtained from the outermost layer. The massive texture of the innermost layer may reflect a rapid growth. Further study of the intermediate layers is needed to verify the hypothesis.

The discrepancy between the two growth rates suggests an irregular rate of accretion not considered in previous work¹². This hypothesis is supported by the nature of the surface texture and by the presence of coccospheres. As the accretion rate of marine Mn nodules is considered to relate intimately to low sedimentation rates and to the water current on the sea floor¹⁵, the drastic change of rate may correspond to a change in the deep-sea environment in the region during the late Pleistocene.

If the assemblage of microfossils in the layers of Mn nodules can be correlated with those in the sedimentary sequence of the same region, more information on both the origin of the nodules and on the regional palaeoenvironment of the deep sea should be provided.

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Climatic implications of stable carbon isotopes in tree rings

STABLE carbon isotope variations in tree rings are normally attributed to either a direct temperature dependent fractionation process in the tree¹, or alternatively to variations in atmospheric composition^{2,3}. To clarify this apparent conflict we have begun measurements of ratios in rings of Southern Hemisphere trees. In spite of some obvious differences between the two trees studied so far, both show a positive correlation with regional temperature trends over the past 75 yr. This result is discussed in terms of the alternatives above, and with the view of extracting palaeoclimatic information. Verification of such influences could provide > 2,000 yr of data on the climate of the region.

Five-year blocks were taken from two King Billy pines (*Arthrotaxis selaginoides* D. Don) which grew within 200 m of each other in natural forest in mountains of northern Tasmania (Fig. 1). A total of 71 separate burns were made on the 25 5-yr blocks of one tree and the CO₂ from each burn analysed at least twice. The δ^{13} variations for each block were generally < 0.1‰. The second tree had 31 5-yr blocks and 50 burns were performed. A full discussion of the experimental technique, tree environment and dendrochronology, and raw data will be published elsewhere.

Variations of 0.2–0.3‰ in δ^{13} are observed in consecutive blocks but these are not always duplicated in the two trees. On a longer time scale, however, similar major trends are evident in both trees, a decrease in δ^{13} from 1895 to ~ 1945 and a rapid increase back to the 1895 level by 1970. This is

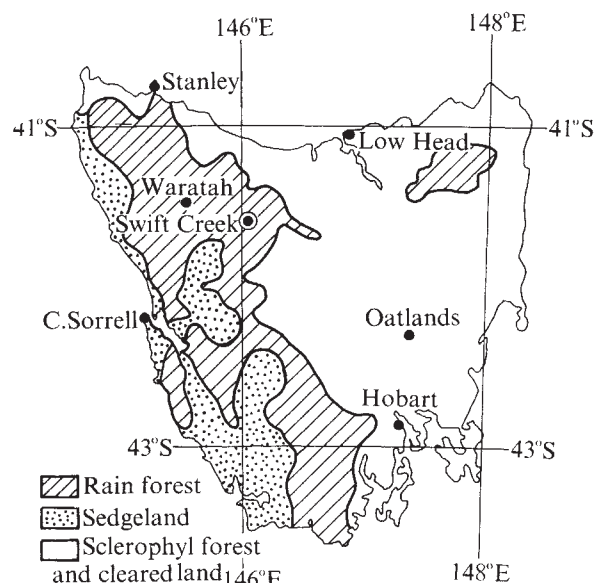


Fig. 1 Map of Tasmania showing the location of the six climatological stations and the Swift Creek site from which the study trees were obtained. Representative samples of 25–75 mg from each block were burnt in an oxygen atmosphere similar to that described in ref. 4. The resulting CO₂ was cryogenically trapped and its isotopic composition analysed using a Micromass 602B mass spectrometer. Results are reported in conventional δ^{13} units, where

$$\delta^{13} = \left[\frac{(^{13}\text{C}/^{12}\text{C})_{\text{wood}}}{(^{13}\text{C}/^{12}\text{C})_{\text{standard}}} - 1 \right] \times 1,000(\text{‰})$$

A cylinder of CO₂ was used as a standard and by adding an arbitrary –8.0‰ our data were made comparable to the PDB standard. The influence of solvent extraction, cellulose separation and variations in oxygen isotopes were found to be of similar magnitude to those reported elsewhere^{5–8}, relatively constant throughout heart and sapwood, and incapable of significantly influencing the long term trends observed. Thus the bulk of the measurements were on whole wood samples.

illustrated in Fig. 2 which gives δ^{13} histograms for each tree. The large block-to-block variations have been partly suppressed with a 3-block (15-yr) running mean.

Corresponding 5-yr blocks (means) of ring width, rainfall and temperature data have been analysed and correlated with the δ^{13} curves. The only significant correlations (5% significance level as indicated by the Student's *t* test) are with maximum temperatures. The correlation coefficients improve with the 3-block smoothing, and also consistently and significantly as the temperature data are restricted to summer months, particularly February. Convective mixing in the lower atmosphere during the daytime ensures that maximum temperatures are far less susceptible to local influences than are minimum (and thus mean) temperatures. Thus maximum temperatures will be more influenced by, and therefore more highly correlated with, the mean temperature of a larger volume of the atmosphere, particularly in summer, when convective activity is greatest. It may also be important that January and February maximum temperatures more closely represent times of tree growth. In Fig. 2 the pecked curve represents the 3-block running mean of February maximum temperatures for six stations surrounding the tree location (Fig. 1). The consistent long term behaviour of all stations (and, by implication, the tree site) is indicated by the shaded area which encompasses the standard deviations obtained by averaging the normalised deviations from the mean for each station. Similar temperature trends are suggested by data of Coughlin⁹ for 9 provincial towns in SE Australia, by sea surface measurements east of Tasmania (D. J. Rochford, personal communication), and also by recent data for New Zealand¹⁰.

A regression analysis of δ^{13} against temperatures for the curves of Fig. 2 gives temperature coefficients of 0.48 ± 0.12 and $0.24 \pm 0.11\% \text{ } ^\circ\text{C}^{-1}$ for trees B and A respectively. The correlation between unsmoothed temperature and mean tree data is significant to the 1% level.

A temperature dependent fractionation process during photosynthesis with a theoretically derived coefficient of $0.36\% \text{ } ^\circ\text{C}^{-1}$ has been proposed by Libby¹², and supported by a comparison between the δ^{13} trends in a German oak and Western European temperature variations. The comparison is far less convincing than those in Fig. 2 and gives a temperature coefficient which is now known to be overestimated⁷. Laboratory investigations of a temperature fractionation effect in

non-arboreal species^{2,11} generally show an effect very much smaller and of opposite sign to those reported here.

Freyer and Wiesberg^{2,13} have summarised their own extensive measurements⁷, plus those of Farmer and Baxter³, relating to δ^{13} values in Northern Hemisphere tree rings. From trees selected as representing carbon exchange with the free atmosphere, a common δ^{13} pattern has emerged. It falls $\sim 0.5\%$ between 1905 and 1940, rising $\sim 0.3\%$ by 1960 and falling 0.3% thereafter. Both sets of authors propose that the trees are registering variations in the isotopic composition of the atmosphere. Dilution of atmospheric ^{13}C by the release of biospheric CO_2 accompanying increased land cultivation is identified with a sharp fall early in the century, and fossil fuel combustion accounts for only a small overall decrease. Climatic variations possibly acting through ocean and biomass exchange are mentioned as possible contributing factors. Correlation coefficients between the tree ring data of Fig. 2 and those described above are ~ 0.5 , and scarcely significant at 5%. Over the years 1905 to 1940–50, however, the similarity is more pronounced.

Much of the δ^{13} variation and the similarity could be accounted for by CO_2 exchange with the temperate oceanic mixed layer as the result of temperature changes. Freyer and Wiesberg² calculate a change in δ^{13} in the atmosphere of $+0.3\%$ for each 1°C temperature increase. The exchange would be dominated by long term temperature variations in the Southern Hemisphere whose oceans contain 80% of the total temperate mixed layer. The regional temperature variations shown in Fig. 2, however, may not be representative of the hemisphere because of a lack of spatial coherence of temperature variation on a sub-hemisphere scale¹⁴. For the same reason, we cannot dismiss the possibility that similarities in δ^{13} variations in both hemispheres may have resulted from the fortuitous occurrence of similar temperature trends in the regions from which trees were collected. The Freyer and Wiesberg and the Farmer and Baxter trees may have responded directly to regional temperature trends which were different to those accepted for the Northern Hemisphere as a whole¹⁵.

Whatever the cause, the tree analyses reported here show a significant correlation with regional temperature on a time scale longer than 5–10 yr. We are hesitant to accept a single explanation in terms of a direct temperature effect on photosynthesis, because on occasions changes in δ^{13} differences between the two trees would require up to 2°C changes in the 5-yr averages of the temperature differences between sites 200 m apart. It is possible that the local canopy significantly influences the isotope ratio either by temperature effects of shading or by an isotope enrichment process as discussed by Keeling¹⁶, with indirect temperature links. It is anticipated that information from a wider selection of trees, on both longer and shorter time scales, will resolve these questions.

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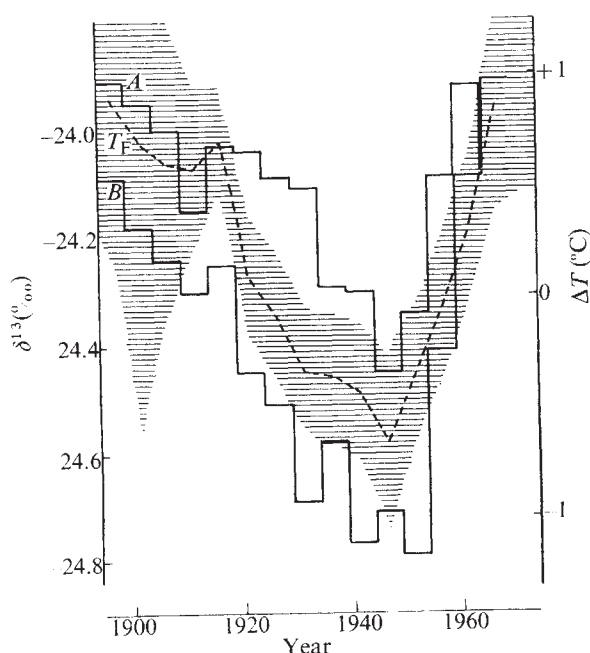
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Fig. 2 Smoothed deviation (ΔT) from the long term average of February maximum temperature for six Tasmanian stations and the δ^{13} of trees A and B.



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Spontaneous measurement by young children

WHETHER or not the young child can make transitive inferences ($A > B$, $B > C \therefore A > C$) is still a controversial issue. Bryant and Trabasso¹ have claimed that he can, provided that he remembers the necessary information, but this claim has been disputed^{2,3}. Youniss and Furth's main argument is that it is one thing to show that a child can make inferences, but quite another to demonstrate that he puts these inferences to any effect. They cite the measurement experiment of Piaget *et al.*⁴ as evidence that the child does not make inferences spontaneously to solve problems. Here children had been shown a tower of bricks on a table and asked to build one as big on the floor. A stick the same height as the first tower was available: the question was whether the child would use this as a measure to equate the towers, thus making an inference ($A = B$, $B = C \therefore A = C$). Children below 8-yr old did not measure with the stick, and it was concluded that they had no idea of measurement. We here report experiments which lead to the opposite conclusion.

An alternative interpretation to Piaget's is that children do understand that it is possible to use intervening measures, but did not in this case realise that a direct comparison of the two towers by eye might be unreliable and thus that some measurement was needed. We decided to test this notion in three experiments in which, as the two quantities to be compared were invisible, it was very obvious that a direct comparison was not viable. We predicted that here children would measure.

Our experimental test involved 4 trials; in each the child was given two identical looking black wooden blocks (8 inch high, 3 inch wide) side by side on a table. Each had a hole in the top, the complete depth of which could not be seen: the hole was either 6 or 4 inch. In 2 of the 4 trials the holes were the same (6 inch with 6 inch, 4 inch with 4 inch): in the other two one hole was 6 inch, the other 4 inch.

Between the two blocks lay a 10-inch long stick: its central 2-inch portion was yellow while the surrounding 4 inch at either end was red, so that the yellow portion was uncovered when placed in a 4-inch hole and covered in a 6-inch hole.

In each trial the child was asked to find out whether the two holes were the same and, if not, which was the deeper. We wanted to know if the child would spontaneously use the stick as a measure to make this comparison. If he did not on any trial he was given a prompt to use the stick as a measure.

In experiment 1, 20 5-yr olds and 20 6-yr olds were also

told on each trial to use the stick though they were not told how. In experiment 2 the same number of 5 and 6-yr olds were told nothing about the stick. In experiment 3 the same number of 5 and 6-yr olds were given the same task as in experiment 2, except that the entrance to the two holes was widened and painted white, to see if this would distract the child from measuring. (The depth of the holes was still invisible.)

Also in each experiment each child was given a pre- and post-test version of Piaget *et al.*'s measurement task, which was exactly the same except that it was miniaturised, with a toy table and towers and sticks which were either 6 or 4 inch.

All the children failed both traditional tower measuring tests in all three experiments. Many of them were, however, successful in our hole measuring task. As Table 1 shows, the majority of children used the stick as a measure without any prompting, and most of their judgments were correct. A substantial number of children were never prompted and were correct on every trial. There were no significant differences between age groups or experiments. We conclude that young children do have an idea of measuring.

In experiment 4 we tested our idea that a crucial factor is whether or not a child realises that a direct comparison will be unreliable with 40 5-yr olds and 40 6-yr olds. Half had to compare holes in Perspex boxes. To make direct comparisons of the visible holes unlikely we changed the depth of the holes in this experiment to 6 and 5.5 inch and we placed the blocks on different tables in both conditions. Otherwise the procedure was the same as before.

The prediction was that Perspex boxes, by encouraging direct comparisons, would lead to less measurement. Although the results (Table 1) provide some partial support for this prediction, the difference was not significant. Many children still measured even in this experiment.

Whatever is the importance of the child's appreciation of the precariousness of direct comparisons, we have established contrary to all preceding evidence that young children can spontaneously use intervening measures. This result has some educational significance and since the measuring must have involved inferences it is strong support for the claim that young children make, and benefit from making, transitive inferences.

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Table 1 Performance in the four experiments

	Expt 1		Expt 2		Expt 3		Expt 4 Wooden box		Expt 4 Perspex box	
	5 yr	6 yr	5 yr	6 yr	5 yr	6 yr	5 yr	6 yr	5 yr	6 yr
Mean age (yr. months)	5.6	6.6	5.6	6.5	5.8	6.4	5.5	6.6	5.7	6.8
No. of children (out of 20) measuring on every trial with no prompt	17	19	15	14	16	16	11	16	7	14
No. of children never prompted and correct on all 4 trials	10	15	10	9	11	10	8	7	2	7
Mean no. (of 4) of correct judgments by those children never prompted	3.41	3.79	3.53	3.43	3.38	3.50	2.84	2.91	3.07	3.44

Territory size, breeding time and mating preference in the Arctic skua

THE Arctic skua is a polymorphic seabird with three phenotypes in plumage—pale, intermediate and dark. The dates during the breeding season when new pairs breed are related to the phenotypes of the males^{1,2}: on average, dark males breed before intermediate males who breed before pale males. This effect is most pronounced when the male has no previous breeding experience³. These observations can be explained by assuming that females have mating preferences for the darker males^{2,4}. Each breeding pair of Arctic skuas defends a territory: some defend their territories very strongly, attacking intruders viciously, others seldom attack. In the red grouse, androgen levels have been shown to determine both aggression and mating success of males⁵. We show here that the size of a male's territory may either correlate with breeding time and mating preference through a common cause, or determine them both directly.

Since 1973 we have studied the colony of Arctic skuas on Fair Isle. This study has included the accurate surveying of the positions of the nests of all pairs of birds in the colony. We have mapped the nests on paper, drawn the lines connecting each nest with its adjacent nests and then the lines perpendicular to, and bisecting, each of the connecting lines. This divides the area of the colony into polygons, each enclosing a nest. Parts of the colony are occupied by great skuas as well as Arctic skuas. The great skuas' nests were also mapped, since great and Arctic skuas show inter-specific territoriality. We consider the area of each polygon to be a measure of the size of the territory. When territories are sharply bounded in a dense colony, they naturally tend to become polygonal⁶. Only pairs with territories surrounded by others were included in the following statistical analysis. The full details of this method of mapping territories and the statistical analysis will be described in a subsequent paper.

In the data from those males who are mating with a new female and who have previous breeding experience, the hatching date is negatively correlated with territory size. The larger the territory, the earlier the breeding date. The correlation is very significant statistically (see Fig. 1: $r = -0.462$; $N = 35$; $P < 0.01$). There is no significant correlation if the males are without previous breeding experience ($r = -0.182$; $N = 33$), and no correlation at all if they are breeding with the same female as in previous years ($r = 0.015$; $N = 100$). The overall correlation for males breeding with a new female, however, is very significant ($r = -0.334$; $N = 68$), and the difference in the correlation for these males is not significant whether they have previous breeding experience or not ($\chi^2 = 1.544$).

Available evidence and casual observation at the colony

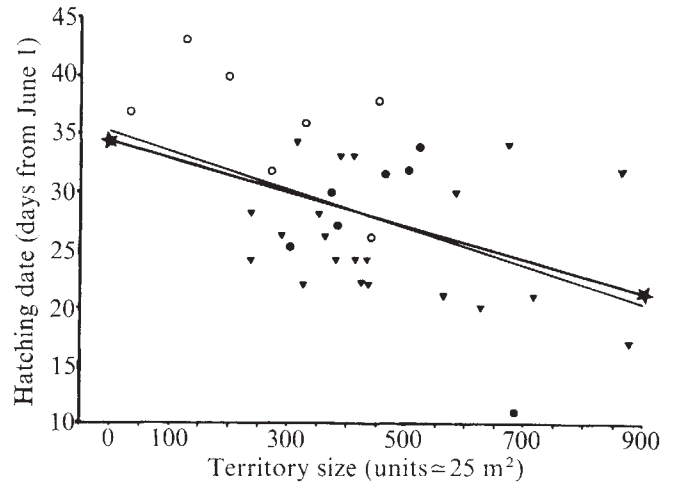


Fig. 1 Relationship between territory size and breeding time among males with previous breeding experience who are mating with a new female. A computer simulation, assuming that females have probabilities of mating proportional to the sizes of territories of males who are still unmated, gives the regression $y = 34.48 - 0.01455x$ (★—★) which is very close to the observed regression $y = 35.52 - 0.017x$ (—); ● Dark; ▼ intermediate; ○ pale.

suggest that the pairing of males with some previous experience of breeding may be different to the pairing of those with no experience. All the experienced males who changed their mate, bred on the same territory as they had done in previous years. Their new mate was often a female who had also bred nearby in the past. Immature males may find their mates differently, however. The immature birds return to the colony in the years before breeding, forming loose groups, or 'clubs', in particular parts of the colony. The inexperienced males probably select their mates from among the females of the club having taken over a territory during the year before breeding. Since the experienced males start the season with a territory, breeding time is a good measure of the time taken to find a mate. The inexperienced males, however, may have already paired with a female and defended a territory before breeding in the following year: if so, they would not form a group comparable to experienced males seeking a new mate, and breeding time would not be so closely related to territory size; the correlation is in fact reduced from -0.462 to -0.182 , but, as we have already stated, this reduction is not significant given our present sample sizes.

With the data available, we can only speculate on the cause of the correlation in the males between breeding time and territory size. The following hypotheses would explain the observations:

(1) Females may simply prefer males with larger territories. We cannot see how this hypothesis can be tested.

Table 1 Fitting of parameters of negative assortative mating to the data of Murton *et al.* on matings between phenotypes of feral pigeons

Parameters fitted	Values of the fitted parameters at maximum likelihood			Values of χ^2	Degrees of freedom
None	—			50.869	6
α	0.340			40.945	5
β	0.508			26.856	5
γ	0.397			35.243	5
$\alpha \beta$	0.327	0.520		8.271	4
$\alpha \gamma$	0.355	0.416		10.782	4
$\beta \gamma$	0.508	0.376		6.565	4
$\alpha \beta \gamma$	0.000	0.508	0.376	6.565	3

In the model, the females have preferences α , β and γ to mate with the phenotypes 'wild type', 'blue checker' and 'dark-blue checker'. The preferences are expressed only when the male's phenotype is different from the female's. Also included in the data were rare birds with other phenotypes. These were grouped together as 'others' and it was assumed there were no female preferences in their favour.

(2) Territory size may determine both breeding time and mating preference. Females will be more likely to land by random choice on the larger territories. The male with the larger territory will therefore pair earlier in the season. Such an effect would certainly account for the correlations we have observed. Given the data of Fig. 1, we have computed for each female the probabilities of mating proportional to the sizes of territories of males who are still unmated. This gives a correlation coefficient of -0.393 which is not significantly different from the observed correlation. Unfortunately this simple and satisfying hypothesis cannot be the complete explanation of our observations: as we have already said, when males change their mates, the females usually come from nearby territories and not at random. Nonetheless, some of the females may be arriving to land on the territories at random, and if the darker males have larger territories this would partly account for their earlier average mating time and the mating preference in their favour. Sexual selection would thus favour increased territory size since earlier pairs have greater reproductive success.

(3) Territory size and breeding time may both be determined by a common cause. Territory size may be a measure of the intensity of breeding behaviour. It would then be related to the mating preferences of females for the different phenotypes of males. O'Donald, Wedd and Davis² and O'Donald⁴ estimated the mating preferences for intermediate and dark males according to a number of specific models of mating preference. The best estimates suggested that $\sim 29\%$ of females preferred intermediate males and 18% preferred dark males: these were the values that gave the closest fit by maximum likelihood. On Fair Isle, intermediates mate assortatively. A model of assortative mating gives an estimate of the mating preference for intermediates which agrees with that obtained from the distributions of breeding times^{7,8}. The mating preference for dark males is only slight and not statistically significant, but this is perhaps to be expected in view of the small number of dark males in the sample. If the mating preferences are determined by sex drives of the males, we should expect that the intermediate and dark males would also have the larger territories; they do, but not significantly so, for our present sample contains only few pale males (see Fig. 1). Our data are fully in agreement with the expectations of this hypothesis but to test it more rigorously further observations are required, both on territory size and its changes among pale, intermediate and dark males, and on the behaviour in the clubs of immature birds.

No correlation has been observed between territory size and breeding time in pairs that have bred together in previous years. There is also very little variance in the breeding dates of particular pairs in successive years⁹. The large territories tend, however, to get smaller and the small territories tend to get larger ($r = -0.698$; $N = 21$; $P < 0.001$). This could easily be explained if territories in successive years were random samples of available territories. Territories are not, however, allotted each year at random: the same pair always keep the same territory, whose size is not expected to vary since the average territory size in a particular part of the colony does not vary much from one year to the next. Perhaps larger territories take more defending since they are more likely to be disturbed by intruders. If the effort of defending a territory has evolved to an optimum by natural selection, a pair with a larger territory may, therefore, allow it to contract during the breeding season and so start with a smaller territory next year.

An interesting comparison between melanism in the Arctic skua and melanism in feral pigeons gives further support to our third hypothesis. Murton, Westwood and Thearle¹⁰ have shown that the melanistic blue checker and

dark-blue checker pigeons have larger testes during the breeding season and remain longer in breeding condition than wild-type pigeons. Pigeons show negative assortative mating of their phenotypes, however, unlike the positive assortative mating of the Arctic skua. We have analysed these data using a model of negative assortative mating. In the model, the females have preferences α , β and γ to mate with wild-type, blue checker and dark-blue checker males. These preferences are not expressed when the males have the same phenotypes as the females. (In the model of positive assortative mating for the Arctic skua⁷, the mating preferences are expressed only when the males have the same phenotype as the females.) Table 1 shows the result of fitting our model to the data of Murton *et al.* The females clearly prefer the melanistic males. In pigeons, this is likely to have a physiological explanation since the larger testes of the melanics imply higher levels of gonadotropins and more intense sexual activity. It would be interesting to see if there are similar physiological differences between melanistic and non-melanistic Arctic skuas. Such differences would explain both mating preferences and the correlation between territory size and breeding time, but would not explain the assortative mating. Positive assortative mating can evolve as a result of linkage disequilibrium between the genes for the preferences and those for the preferred phenotypes¹¹. Negative assortative mating could evolve only as a result of some specific selective advantage.

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Retrograde amnesia following electroconvulsive therapy

AMNESIC agents, such as electroconvulsive stimulation, can cause loss of memory for events that occurred before treatment¹. Usually as the interval between learning and convulsive treatment is increased, the resulting retrograde amnesia is diminished^{1–3}. This temporal gradient of retrograde amnesia can sometimes cover several years⁴. Clinical descriptions of the amnesic syndrome suggest that information about the temporal sequence of events can be more severely impaired than other aspects of memory^{5,6}. Thus, an amnesic patient may describe a past event accurately but be unable to report when the event occurred. We have administered a new remote memory test based on former one-season television programmes to psychiatric patients receiving bilateral electroconvulsive therapy (ECT), and report here that memory for temporal order is markedly affected by ECT. ECT caused retrograde amnesia for order information about programmes broadcast from 1 to ~ 7 yr before treatment, but not for programmes broadcast from 8–17 yr before treatment.

The subjects were 17 female and 3 male psychiatric in-patients 27–64 yr old (mean = 45 yr), for whom a course of bilateral ECT had been prescribed for relief of depressive

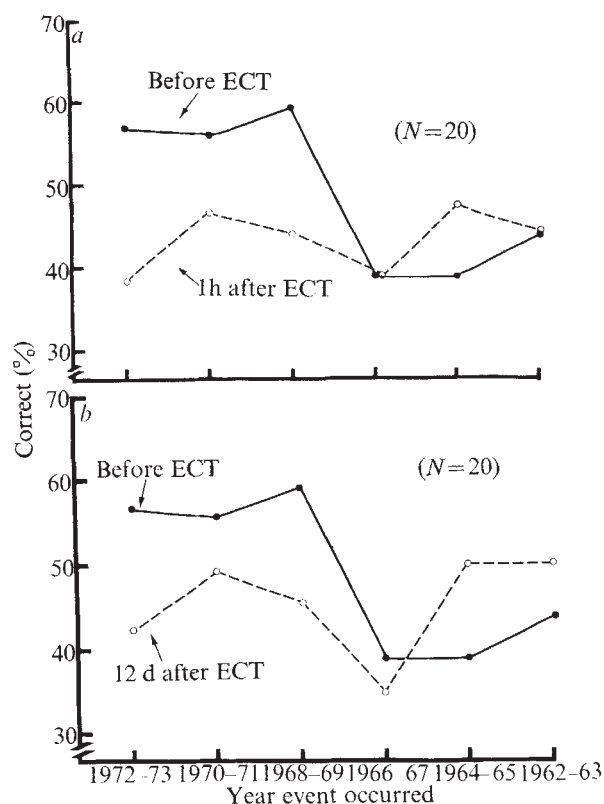


Fig. 1 Results of the test requiring temporal judgments about remote events administered to depressed psychiatric patients receiving a course of bilateral ECT. Testing was conducted before ECT, 1 h after the fifth treatment (a), and 1–3 weeks (mean = 12 d) after the completion of treatment (b). The test asked patients to select from sets of 3 former television programmes the one that was broadcast most recently. Comparisons were always between a programme broadcast for one season between 1962 and 1973 and two programmes broadcast for one season 5 years earlier. The time indicated is that when the correct choice was on the air. Values >42% are significantly above the chance level of 33% ($t > 2.1$). ECT selectively impaired performance for the time period 1968–1973.

illness. Four of these 20 patients had received one course of ECT from 3 months to 14 yr previously. ECT was given with a Medcraft (B-24) machine (140–170 V, 0.5–1.0 s), with temporal-parietal electrode placement. This treatment was given three times per week on alternate days under barbiturate anaesthesia (methohexital sodium, 60–120 mg), and atropine (0.8 mg), with full muscular relaxation (succinylcholine, 20–60 mg).

The temporal judgment test was based on previously described recognition tests^{7,8} involving the names of 85 one-season television programmes, 5 from each year 1957–1973. Popular exposure of the programmes from each year was relatively similar, and names were apparently learned close to the time the programmes were on the air. A three-alternative, multiple-choice test of 36 questions was constructed that repeated the question: "Which television programme was on the air most recently?" The correct answer was always the name of a programme that was broadcast for one season from 1962 to 1973. The two incorrect choices were the names of programmes broadcast 5 yr before the correct choice, 1957–68. The order of the 36 questions was random with respect to the periods they covered. All patients took this test 12–18 h before their first treatment, 1 h after their fifth treatment, and 6–25 d (mean = 12 d) after completion of their course of ECT (5–18 treatments, mean = 9.0). Testing was conducted from October 1974, to August 1975.

Figure 1 shows performance on the temporal judgment test before and after ECT. Before ECT, patients performed

similarly to a control group⁸ of 30 hospital volunteers ($F = 0.1$, $P > 0.3$). Apparently performance on this test was not noticeably affected by depressive illness. One hour after the fifth ECT (a), the performance was markedly impaired. The effect of ECT on 1968–73 scores accounted for 90% of the variance in before–after scores, and the before–after difference was significant for 1968–69 ($F = 4.8$, $P < 0.05$) and 1972–73 ($F = 7.0$, $P < 0.05$).

The results cannot easily be explained by gross confusion or by an inability to obtain high test scores after ECT. First, verbal IQ is normal by 1 h after 5 bilateral treatments^{4,9}. Second, ECT did not simply reduce the frequency of high scores across all time periods. For the three periods not affected by ECT (1962–1967), patients obtained 15 scores of 67% or better before ECT and 14 scores of 67% or better after ECT. Thus, the impairment after ECT cannot be attributed to a global impairment in the ability to perform well, but is consistent with the interpretation that ECT selectively affected memories acquired in recent years. This conclusion is also supported by the fact that after ECT the score for 1972–73 was lower than the score for any other time period and was not significantly above the chance level of 33% ($t = 0.9$, $P > 0.3$).

Figure 1b shows that the impairment in temporal judgment for remote events persisted 1–3 weeks after completion of treatment. At this time the effect of ECT on 1968–1973 scores accounted for 71% of the variance in before–after scores, and the before–after difference was significant for 1968–69 and 1972–73 ($F > 9.0$, $P < 0.01$). A comparison of performance 1 h after the 5th ECT with performance 1–3 weeks after the completion of ECT indicated that no recovery had occurred during this period. The scores were nearly identical on these two occasions ($F = 0.7$, $P > 0.3$) and did not differ by >6% for any period.

These results indicate that ECT markedly disrupted the ability to make temporal judgments about remote events. Compared to amnesia for the names of former programmes⁴, retrograde amnesia for temporal information about these programmes was more extensive and persisted for a longer time after treatment. Thus, amnesia for names of former programmes covered the past 1–3 yr, whereas amnesia for temporal information covered ~1–7 yr. Memory for the names of past programmes had recovered by 1–2 weeks after a course of ECT (mean = 7.6 treatments)⁴, whereas judgment of temporal relationships between these programmes was still impaired 1–3 weeks after ECT (mean = 9.0 treatments). A long term follow-up study is now in progress to determine how long after ECT the disturbance of judgment remains.

Our results, together with previous experimental findings^{4,10} and clinical case reports^{11,12}, support Ribot's hypothesis¹³ that the resistance of memory to disruption increases with time. These results differ from findings that ECT⁸ or Korsakoff psychosis¹⁴ caused retrograde amnesia for past public events that affected all past time periods almost equally. Ageing also appears to affect memory for past public events equally across all time periods, rather than to cause a greater loss of memory for more recent time periods^{15,16}. These studies do not, however, provide strong evidence for or against Ribot's hypothesis. First, the performance of Korsakoff patients¹⁴ was so close to chance for questions about past time periods that a tendency for performance to be affected differently could easily have been masked. Indeed, in a subsequent study using easier questions, Korsakoff patients did exhibit a selective retrograde amnesia for more recent time periods¹⁰. Second, in the study of remote memory it is difficult to compare results for different times unless efforts are made to eliminate possible sources of sampling bias¹⁵. Third, performance of normal subjects was usually about the same for all times (1930–1969)^{9,15}, possibly because the absence of questions about the most recent years precluded the appearance of a

forgetting curve. In contrast, normal performance on questions about former television programmes, including questions about the immediately preceding years, was much better for recent periods than for more remote periods^{7,8}. One interesting possibility is that the development of resistance to amnesia parallels the course of normal forgetting. By this view, differential resistance to amnesia should not be demonstrable in the asymptotic limb of a forgetting curve.

Taken together, the evidence supports the conclusion that the resistance of memory can develop for several years after learning, and that temporal order information is particularly vulnerable to disruption. The neural substrate of memory may change over the years so that material in memory becomes both harder to recall and more resistant to disruption.

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Development of visual stimulus-seeking in dark-reared kittens

EARLY visual experience is important for the development of vision in kittens. The response specificity of visual neurones for different environmental features can be affected by selective visual exposure to those features, and leads to long term changes in visual capacities¹⁻⁴. Modification occurs only if the kitten is exposed selectively during the first three months of life. The peak period of sensitivity for "tuning" seems to occur between days 28 and 35, and thereafter declines rapidly; by the end of the second month, neuronal connections are less readily modifiable⁵⁻⁶. The peak of the sensitive period coincides with the clearance of the ocular media⁷, the beginnings of adequate locomotor control (M. S. Levine, C. D. Hull, and N. A. Buckwald, unpublished), completion of the most active phase of myelination in the optic nerve and optic tract (C. L. Moore, R. E. Kalil, and W. A. Richards, unpublished), and the period of greatest synaptic density in the visual cortex⁸. In the present study we show that kittens do not spontaneously seek light at this time. Rather, there is a sudden onset of light-seeking behaviour at two months of age, when the cortical contour-coding system is not very sensitive to environmental modification, and many other adult visual characteristics have already been acquired.

Beginning at 5 d of age (before the eyes are open), six kittens taken from three litters were housed with their mothers in large nesting cages in a completely dark room where they remained for 3 months. From day 15 for 1 kitten, day 20 for

Table 1 Pattern of self-induced exposure to visual stimulation in light- and dark-reared kittens

	Short presses (<0.5 s)		Long presses (≥ 9.5 s)	
	40-49 d	70-79 d	40-49 d	70-79 d
Dark-reared kittens				
1	0.804	0.337	0.000	0.120
2	0.750	0.285	0.000	0.169
3	0.523	0.339	0.000	0.095
4	0.877	0.382	0.000	0.081
5	0.492	0.340	0.000	0.052
Mean	0.689	0.337	0.000	0.103
Light-reared kittens				
1	0.792	0.746	0.000	0.000
2	0.605	0.569	0.009	0.014
Mean	0.699	0.658	0.005	0.007
Auditory control				
Tone	0.500	0.267	0.024	0.000
Light	0.498	0.289	0.043	0.167

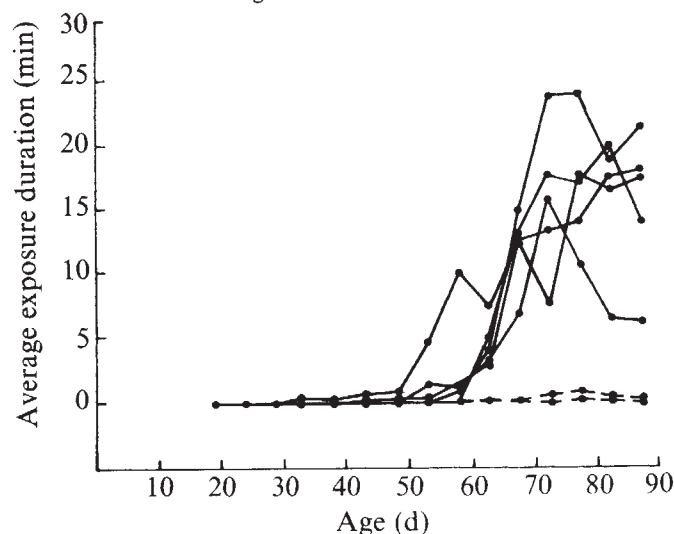
Proportion of short and very long presses within 10-d blocks before and after onset of light seeking.

2 and day 23 for 3 kittens, to day 90, the kittens were transported in a light-tight box and placed individually each day in an experimental chamber, 76 cm wide by 91 cm long. A cylindrical tunnel, 6.5 cm in diameter and 34 cm long, extended from the rear of the chamber. A transparent Plexiglas panel, measuring 10×10 cm and recessed 5 cm, was positioned in the near end of the tunnel. A pattern of stripes subtending a visual angle of 15° was mounted at the far end of the tunnel and was illuminated continuously when the panel was pressed. Different cats were exposed to stripes of different spatial frequencies, but this did not seem to influence the pattern of responding. The tunnel was maintained at eye level by adjusting the level of the floor to compensate for the kitten's growth.

At the beginning of each session the kitten's nose was pressed against the panel several times for a total of about 5 s of stimulation. In addition, after the kitten had been left alone in the chamber, the light was flashed briefly to signal the position of the panel. Subsequently, the kitten could obtain light only by pressing the panel.

Two light-reared controls were treated identically except that they were housed in a larger cat colony maintained on an alternating 12 h light-dark schedule. One of the kittens reared in the dark served as an auditory control. It was allowed access to light only on alternate days. During the sessions every other

Fig. 1 Minutes of self-produced exposure per session, averaged over 5-d periods, expressed as a function of age of the kittens. Solid lines represent individual dark-reared subjects; broken lines, light-reared controls.



day, pressing the panel turned on a tone (maximal energy between 3,000 and 4,000 Hz) instead of the light.

Four of the dark-reared kittens and both light-reared kittens were given two 1-h sessions daily. The auditory control and one other dark-reared kitten were given two sessions per day until day 70 and thereafter were run only once per day.

Figure 1 shows minutes of self-produced exposure per session, averaged over 5-d periods, for all the animals except the auditory control. Neither dark- nor light-reared kittens press for much light in the first two months of life. Beginning at about day 60, however, the dark-reared kittens show a dramatic increase in self-produced exposure to light, in sharp contrast to the controls.

The increase in exposure is accompanied by a change in the pattern of responding. Both the number of presses and the average press duration increase. Before day 60, the typical mean press duration rarely exceeds 1.0 s (median rarely exceeds 0.5 s), but afterwards it seldom falls below 3.0 s (median between 1.0 and 4.0 s). Shorter presses and brief "knocks" become much less frequent, and some very long presses are produced. Before day 60, a press of 5 s or longer is rare, but afterwards presses of 1–3 min are not uncommon. Presses of up to 8 min were recorded on several occasions. Table 1 shows the shift in pattern of pressing for each group. Although there is a marked difference between groups, the pattern is consistent within each group.

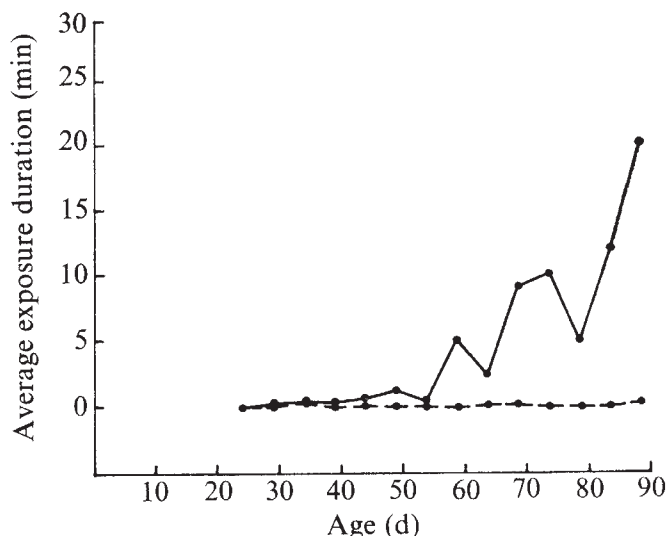


Fig. 2 Minutes of self-produced exposure to light (solid line) or sound (dotted line) per session, average over 5-d periods for one kitten.

All the dark-reared animals begin to change their behaviour at about the same age. Perhaps it could be argued that lack of strength or motor coordination prevents the kittens from panel pressing intensively before two months of age. Our data do not support this notion. All the kittens had pressed the panel for brief periods before day 30. There is no gradual increase in the number of seconds of exposure with time nor is any relationship between onset of the behaviour and body weight apparent.

It seems probable that the kittens were seeking visual stimulation specifically, rather than some general increase in sensory input. Figure 2 shows the data for the kitten allowed alternating access to light and sound. Clearly it does not seek the auditory stimulation. Typically, it would press for several seconds at the beginning of the session and then not press at all for the remainder. When light was available, it would press for prolonged periods. Additional control sessions were run for the other kittens in which, for alternate 15-min blocks within the 1-h session, pressing the panel did not illuminate the pattern. With no light contingency, the kittens quickly stopped respon-

ding, even though auditory stimulation was available from a microswitch which operated when the panel was pressed.

As was pointed out, the peak sensitivity for the "tuning" of cortical neurones seems to occur in about the fifth week of life^{9–10}. Why should kittens not attempt to gain much access to light until cortical modifiability has declined? Perhaps they only seek such experience when the visual system is approaching a relatively stable stage, i.e., after the ocular media have cleared and the feature detectors have been tuned^{5–8}.

Our results do not throw light on the functional significance of the onset of light-seeking; the phenomenon does not seem to be related to the development of contour detecting mechanisms, rather it may point to other developmental processes in the visual system. Indeed it has been argued¹¹ that the main sites at which perceptual learning influences the system are probably not at the contour coding level.

There is a great temporal discrepancy between electrophysiological studies of cortical maturation, which place the peak sensitivity of the critical period at one month of age, and the present behavioural evidence, which demonstrates an abrupt, light-oriented behavioural change about a month later. The present results emphasise that the functional tuning of the contour-coding system of the visual cortex cannot simply be equated with the process of perceptual development.

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Effect of constant light on nychthemeral variations in serum testosterone in male *Macaca radiata*

CIRCADIAN and seasonal variations in reproductive functions of mammals have been investigated extensively¹. Serum testosterone exhibits a nychthemeral variation in man^{2–6} and the rhesus monkey^{7,8}. Exposure of laboratory rodents which are nocturnal in habit to continuous illumination causes precocious puberty in immature rats and hamsters⁹, and constant oestrus in cycling rats due to elevated gonadotropins¹⁰. Conversely, prolonged exposure to darkness causes atrophy of testes in the hamster due to suppression of circulating FSH and LH^{11,12}. Relatively less information is available on the effects of light on reproduction in the human and non-human primates. From a study of the effects of constant light on serum testosterone in the male bonnet monkey (*Macaca radiata*), we have found that *M. radiata* shows a nychthemeral variation in serum testosterone.

Adult male monkeys weighing 8–10 kg were lodged in individual cages in rooms measuring 10×10×8 feet in our

primate facility. Artificial cool white light was provided by installing two fluorescent tube lights (40 W, 250 V) at a height of 10 feet. The rooms were not air-conditioned but were provided with a powerful exhaust fan to maintain good air circulation. Normally, lights are kept on in these rooms between 0600 and 1800 (IST). The animals were kept under this light-dark (LD) schedule of 12:12 h for 3 months before being used for experiments. The monkeys were fed pelleted food (Hindustan Lever Monkey food) and vegetables three times a day, water being available at all times.

Blood was withdrawn by venepuncture at 6-h intervals. The monkeys were not anaesthetised during these operations. Testosterone in the serum was estimated by radioimmunoassay according to standardised procedures (see footnote for Table 1 for details).

The results show that *M. radiata* like the rhesus monkey (*M. mullata*) also exhibits a nychthemeral variation in the secretion of serum testosterone with a 6–12-fold increase during the night hours (Table 1). Exposure to constant light for 72 h did not bring about significant change in this pattern. Exposure to continuous illumination for 4 weeks, however, abolished the nocturnal surge, this being restored once the lighting was shifted back to normal schedule of LD 12:12 (Fig. 1). While restoration to normality seems to be a relatively fast process, a minimum of 15 d of continuous illumination was found necessary to abolish the nocturnal elevation of serum testosterone. In two of the monkeys, although there was a reduction in testosterone levels at 2000 due to constant light, the levels at 0800 and 1400 increased over the control by two- to three-fold. The reason for the latter is at present not clear to us.

While in the rhesus monkey no elevation in LH levels have been observed that can be correlated with the nocturnal rise of testosterone⁸, in the human Judd *et al.*⁵ observed that the pulsatile rise of testosterone during the nocturnal period is closely preceded by pulsatile changes in LH levels. But since other workers^{13,14} have not observed a change in amplitude and frequency in LH peaks between day and night hours, they suggest that the nocturnal rise of testosterone may be related to a variability of Leydig cell sensitivity to LH during these hours⁵.

Table 1 Nychthemeral variations in serum testosterone in male *Macaca radiata*

Time of day	Testosterone equivalent ng per ml serum Mean $\pm$ s.e.m. (n = 7)
0200	26.0 $\pm$ 3.90
0800	3.96 $\pm$ 0.69
1400	2.29 $\pm$ 0.26
2000	25.6 $\pm$ 3.72

The procedure adopted for extraction of steroid from serum and radioimmunoassay was essentially similar to that described by us earlier for progesterone¹⁷. 2,4,6,7-³H-testosterone was used as the label and the antiserum used was prepared to testosterone-3-oxime-bovine serum albumin. The antiserum at the dilution used (1:40,000 final dilution) cross reacts significantly only with dihydrotestosterone to an extent of 66.0%. The specificity of the antiserum is adequate for the present study, as the latter aims at measuring the total testosterone equivalents.

The nocturnal elevation of testosterone appears from our study not to be influenced by the presence or absence of females in the vicinity, as the males in the present experiment were housed separately from the females. It should, however, be mentioned that the males in the breeding units exhibited a much higher nocturnal level of testosterone (approximately 1.5–2-fold) than the males used in the present experiment.

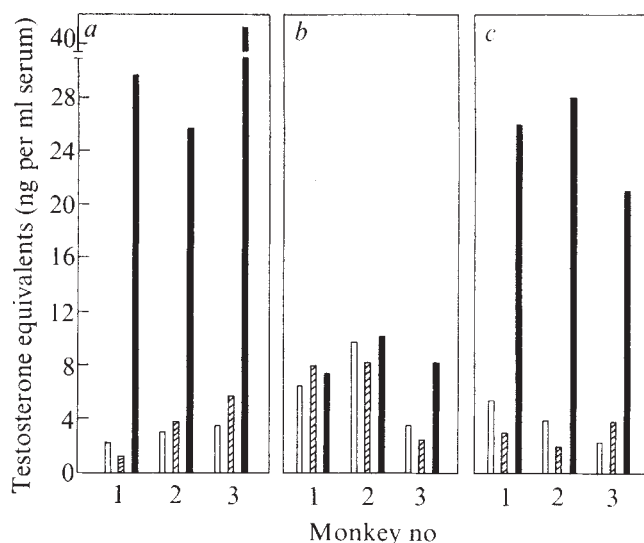


Fig. 1 Effect of continuous illumination on serum testosterone in male bonnet monkeys. Three of the monkeys which showed typical nychthemeral pattern as in Table 1 were selected for this study. a, LD, 12:12; b, LD, 24:0; c, LD, 12:12. Open columns, 0800; hatched columns, 1400; black columns, 2000.

The abolition of nocturnal surge of testosterone in monkeys under constant illumination could be due to either a suppression of gonadotropin secretion or loss of responsiveness of the testis to unchanged levels of gonadotropins.

Interestingly, the monkey differs from the rat in that constant light brings about an increase in pineal hydroxy indole O-methyl transferase (HIOMT) activity resulting in an elevation in melatonin levels¹⁵. Since it is known that many pineal substances (for example, melatonin) are anti-gonadotropic in nature, it is possible that an increase of these factors in monkeys under constant light might be responsible for inhibiting testicular function. The observation that blindness in humans is associated with early gonadal maturation¹⁶ lends support to our suggestion. It should, however, be pointed out that as constant light is a stress, a direct action of the factors associated with this stress on the testis cannot be ruled out. All the same it should be mentioned that no change in the time of sleeping was observed when the monkeys were kept under constant light.

In conclusion, the present study shows that the bonnet monkey exhibits a high level of serum testosterone during night hours, which is abolished on exposure to constant light, suggesting that this variation in serum testosterone is a nychthemeral rhythm.

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Timing of the photoperiod and the hour of birth in rats

THE factors determining the onset of labour are largely unknown, though the regulating mechanisms seem to originate from both the foetus and the mother. The foetus seems to determine the day of birth, through the maturation of the pituitary–adrenal–placental axis, but the mother may provide a fine control, determining the precise hour of birth. Labour varies greatly in its timing and speed of execution, presumably as the result of selection to ensure the maximum probability of survival of both the mother and offspring. Environmental factors such as light, tides and predators are of great importance, providing cues which influence the final hour of birth. Even women, who during childbirth are confined in a most artificial environment, show a marked tendency to deliver in the early hours of the morning^{1–4}.

The reproductive functions of the laboratory rat are linked to the diurnal changes in light and darkness. When subjected to an artificial photoperiod with light from 0500 to 1900, rats of most strains release their ovulatory surge of luteinising hormone from about 1500 on the day of pro-oestrus, and then ovulate between 0200 and 0400 the next morning⁵. Similarly, at the end of pregnancy, a complex series of changes in the plasma levels of various reproductive hormones^{6–12} prepares the animal for labour. As the secretion of these hormones is under hypothalamic control, it may reasonably be asked whether the timing of labour is linked to the photoperiod. The rat gives birth between days 22 and 24 of pregnancy (day 1=sperm-positive vaginal smear), though reliable data, where all extraneous factors have been excluded, are limited. Boer *et al.*¹³ recorded by continuous observation an abrupt increase in the incidence of labour at about 1200 on day 22, with a second and much smaller peak during the early hours of day 23. This study involved some, albeit slight, disturbance of the animals, but the rapid onset of births and their bimodal distribution suggested the existence of an accurate timing mechanism. In the results reported here, we have sought to control the various extraneous parameters which might influence labour, to provide reliable information on the hour of normal delivery. We have also investigated the degree to which the time of delivery is determined photoperiodically.

The hour of birth was recorded in 446 rats of a Wistar strain (laboratory bred from MRC Porton stock); 296 were primiparous, weighing 240 ± 27 g (s.d.) at the time of mating; 87 were multiparous, weighing 268 ± 27 g; and the remainder were of unknown parity. Groups of 80–120 pro-oestrous rats were caged singly with a male from 1600. The following morning, the males were removed and mating confirmed in about 80% of the females by the presence of copulation plugs on the dropping trays or sperm in the vaginal smear. These animals were transferred to single cages until delivery occurred some 22–23 d later. All other stock were removed

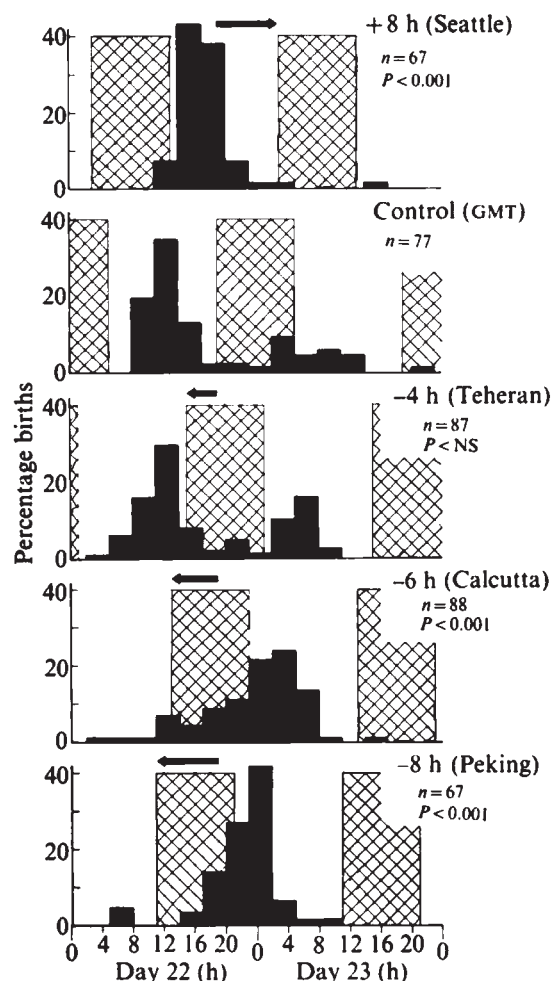


Fig. 1 Effect of the timing of the photoperiod on the hour of birth in rats. The periods of darkness on days 22 and 23 are shown by the hatched areas. The control photoperiod, and the photoperiod of all experimental treatments up to day 12, consisted of 10 h of darkness from 1900 and 14 h of light. Day 12 was lengthened or shortened and the adjusted photoperiod maintained for the rest of pregnancy. The approximate time zones to which, effectively, the rats were transferred are given by reference to the place names. All distributions differ significantly from each other (with the exception of the control (GMT) and –4-h (Teheran) comparison), when compared by either a Kolmogorov–Smirnov or a grouped χ^2 analysis ($P \leq 0.001$).

from the delivery room and the doors were locked. Sufficient food and water were provided so that replenishment was required only once during pregnancy. An artificial photoperiod of 14 h light : 10 h darkness was provided for the controls and experimental groups until day 12, with the lights on from 0500 to 1900 each day. On day 12 only, the various experimental groups were subjected to a longer or shorter day by the retardation or advancement of the onset of darkness. Thereafter, the ratio of light to darkness remained at 14 : 10; so the rats had effectively been transferred to a new “time zone”. The onset of labour was determined by observation every 3 h from 0500 on day 21; the red light of a torch was used during the hours of darkness. This frequency of observation was chosen as a compromise between the need for accurate timing and the need to avoid disturbance. The timing of birth was scored as that 3-h interval within which the first pup was delivered. All dams with litters were removed from the experimental room 6 h after delivery.

The distribution of deliveries in the control group of 77

rats was bimodal, with mean and median time values of 1619 and 1313 (day 22), respectively (Fig. 1). This pattern of deliveries was very similar to that obtained by Boer *et al.*¹³, although the distribution was advanced by about 3 h in all respects. The photoperiod could have been responsible for this time difference, because the animals in Holland¹³ were given 12 h of light from 0700 to 1900, but differences due to rat strain and environmental factors cannot be excluded. A further group of animals ($n=55$) was treated identically to the control group except that it was subjected to continuous darkness from the morning of day 21. The timing of labour was almost unchanged; it was again bimodal with mean and median values of 1519 and 1226, respectively. These times were slightly earlier than those of the controls, but the differences were not significant.

Four groups of rats were subjected to time-zone changes in mid-pregnancy; the results are shown in Fig. 1. The lengthening of day 12 by 8 h (the equivalent of transporting the rats from London to Seattle) delayed the time of expected delivery and created a single distribution with mean and median values of 1710 and 1654 respectively. The reduction of day 12 by 4 h (Teheran time) slightly advanced the onset of the distribution (one animal gave birth before 0500). The second peak on day 23 was more accentuated than in the controls, but the overall difference remained non-significant. The reduction of day 12 by both 6 and 8 h (to Calcutta and Peking time) caused profound changes in the distribution. After the 6-h time shift, the mean and median times for delivery were 2322 and 0044 (day 23); whereas, with the 8-h shift, the mean and median values were 2211 and 2306, respectively. Thus the median time of delivery changed by more than 11 h when day 12 was shortened from -4 to -6 h. Delivery seemed to be transferred from the light period of day 22 to the light period of the next day.

The parity of the mother did not influence the hour of birth. The mean litter size was 10.98 ± 2.68 pups (s.d.) for primiparous rats and 10.92 ± 2.78 for multiparous stock, and there was no correlation between the litter size and the hour of birth when the litters contained six or more young (maximum 17). The mortality rate was 4.2% overall, estimated from the number of dead pups 6 h after delivery, but the true figure was probably slightly higher, as some young may have been eaten without trace. Mortality rose to 39% for deliveries after 1100 on day 23, but most of these births were associated with dystocia. Thus the 12 litters that were produced after 1100 were excluded from the calculations of mean and median delivery times.

These results provide clear evidence that the onset of labour in the rat is determined, in part, by the animal's experience of the timing of the photoperiod in the days which precede labour. As in the study of Mitchell and Yochim¹⁴, rats in the present series exhibited a preference to deliver during the hours of light. In spite of the shifting of darkness through the peak of the control distribution, the rats adjusted their time of delivery such that 82% of all births occurred during the 14 h of light. Although Svorad and Sáčková¹⁵ claimed that the tendency for the mouse to deliver in darkness could likewise be demonstrated by altering the light regime after mating, Porter¹⁶ found that alterations in the timing of the photoperiod had no effect on the distribution of deliveries, so that mice delivered in light as well as darkness depending on a fixed copulation-delivery interval.

In the present experiments, the provision of light *per se* was of no immediate significance, for when the animals were subjected to continuous darkness on the last day the distribution of the deliveries was not retarded; in fact, it was slightly advanced. This suggests that it is the biological rhythm of the animal, rather than a cue from the onset of light or darkness on day 21–22, which determines the hour of birth. As is known for other biological systems¹⁷, these

rhythms take several days to reset when animals are transferred from one time schedule of light and darkness to another, and are not immediately destroyed by the removal of one photoperiodic cue. The daily period of darkness seems to be associated with a physiological change which is inhibitory to labour, but a critical examination of Fig. 1 indicates that this inhibitory effect began to be expressed, as far as deliveries are concerned, 2–3 h before the onset of darkness and it terminated before the lights came on again. Fuchs¹⁸ and Fuchs and Poblete¹⁹ have reported that labour in the rat is presaged by an increase in uterine activity some two or more hours before birth. It is possible therefore that an inhibitory effect on delivery would be influencing maternal physiology 4–6 h before the onset of darkness.

The limits within which normal labour was observed to begin ranged from about 0500 on day 22 to 1100 on day 23, and within this range the median time of delivery varied by up to 12 h according to the timing of the photoperiod. Thus the onset of labour under control lighting occurs relatively early within the normal range, and no photoperiodic change investigated in this study substantially advanced the occurrence of labour. The tail of the control distribution, with its second peak on day 23, seems to be a photoperiodic function, that is, some animals are prevented from delivering on day 22 by the impending onset of darkness at 1900. The advancement of day 12 by 8 h may provide a more satisfactory schedule for some experiments, since this resulted in a single statistically normal distribution, with 82% of the animals giving birth between 1400 and 2000 on day 22.

These results raise the important question of whether the endocrinological changes such as the decline in progesterone titres, the increase in oestradiol and prolactin levels, and the augmented output of prostaglandins, all of which are considered to be involved in preparing the uterus for labour, are retarded or advanced by alterations in the photoperiod. These changes may, however, proceed on an independent time course, so that the precise timing of labour is determined by the arrival or removal of another and as yet undetermined photoperiod-dependent signal, which permits the activation of the already prepared uterus. Experiments are in progress to examine this question.

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Deleterious effect of an anaesthetic on cultured mammalian embryos

THE teratogenic effect of certain tranquillisers on human and animal pregnancies has been known for many years¹⁻³. Anaesthetics and other central nervous system depressants may also be teratogenic when given during early pregnancy, possibly because of the inhibitory action of these agents on mitosis⁴. Anaesthetics can also activate unfertilised mouse eggs *in vivo*, inducing them to develop parthenogenetically^{5,6}. Chronic exposure to low levels of anaesthetics may result in a significant increase in spontaneous abortions and the birth of children with congenital abnormalities^{7,8}. Experiments with rodents⁹ which seemed to demonstrate that chronic exposure to anaesthetics could be teratogenic in early pregnancy have proved difficult to repeat¹⁰, so that doubt has now been cast on the validity of these earlier results. Attention has also been drawn to the possible risk to the human foetus of surgery carried out during pregnancy¹¹. In the experiments reported here we have demonstrated a direct, deleterious effect on early postimplantation rat embryos in culture of a single exposure to different doses of an anaesthetic.

Headfold-stage rat embryos of the CFHB strain were explanted during the afternoon of day 10 of gestation (day of finding sperm in the vagina=day 1 of pregnancy), and cultured *in vitro* for 24 or 48 h in heat-inactivated (56 °C, 30 min) homologous IC serum¹², prepared from blood withdrawn from the aorta of rats anaesthetised with ether. During heat inactivation the bottle containing the serum was opened twice to drive off as much ether as possible. Serum from the same batch was used for both control and experimental culture groups, with Avertin (Winthrop) added to the experimental groups. Except where shown in Table 1, the medium was equilibrated with a gas mixture containing 5% O₂, 5% CO₂ and 90% N₂ for the first 24 h and 5% CO₂ in air for the rest of the culture period. These conditions give optimum development *in vitro* for embryos explanted at the headfold stage (D. A. T. New, personal communication). Since the purpose of the cultures was to test the teratogenic effect of a specific anaesthetic agent, the pregnant females were not killed by etherisation but by cervical dislocation. Where embryos from more than one female were used in one experiment, these were divided equally between control and experimental groups.

In preliminary studies the embryos were cultured in watchglasses or 60-ml roller bottles by New's technique¹³.

With this equipment the gas phase is considerably greater in volume than the volume of the medium. In this instance no teratogenic effect was obtained even with twice the standard anaesthetic dose of Avertin. In subsequent cultures, sealed embryological watchglasses or 12×75 mm plastic test tubes (Falcon, 2003) containing 3 ml and 5 ml of medium, respectively, were used, such that the volume occupied by the gas phase was less than 0.1 ml and 0.5 ml, respectively.

The standard dose of Avertin used to anaesthetise intact rats was used, where 1 ml of culture medium was assumed to be equivalent to 1 g body weight of an intact rat. The standard dose of Avertin used was 0.02 ml of a freshly prepared 1.2% solution of Avertin dissolved in 0.9% saline per ml of serum (or g body weight of an intact rat), equivalent to approximately 240 p.p.m. (by volume). Further groups of embryos were cultured for 24 h in serum containing twice, four times or six times the standard dose of Avertin. Some of these embryos were examined histologically, while others were transferred with the medium to a 60-ml roller bottle containing 2–4 ml of fresh anaesthetic-free serum and cultured for a further 24 h. At the end of culture, at 24 h or 48 h, embryos were examined under a dissecting microscope and an assessment of development was made using purely morphological criteria, such as the presence or absence of a beating heart, closure of the neural tube, the adoption of the foetal position ('turning'), somite number and the yolk sac diameter. Embryos which were not examined histologically were dissected free from embryonic membranes and their protein content was determined microspectrophotometrically¹⁴.

When embryos were cultured with a large gas phase, there was no difference between controls and those cultured in once or twice the standard anaesthetic dose of Avertin for periods ranging from 10 min to 24 h (the results of this and subsequent cultures are presented in Table 1). When the volume of the gas phase was reduced, embryos grown for 24 h in twice the standard anaesthetic dose clearly developed less well (somites indistinguishable, less embryos with a heartbeat) than controls, and four and six times the standard anaesthetic dose had an even greater effect. When the culture period was extended to 48 h and the protein content of the embryos was estimated, there was a significant difference ($P<0.0025$) between control embryos and those cultured in twice the standard dose of Avertin.

The reduction in volume of the gas phase also resulted in a significant difference ($P<0.0025$) in protein content between control embryos and those grown in the standard

Table 1 Effects on early postimplantation rat embryos of a single exposure to different doses of Avertin

Dose of Avertin (multiples of standard dose)	Exposure to Avertin (h)	Total culture time (h)	Culture conditions	Total no. of embryos	Protein content of embryo (µg) mean ± s.e.	Mean yolk sac diameter (mm)	Heartbeat		Average somite no.	Neural folds		Turning	
							Present	Absent		Closed	Open	Complete	Partial or none
1	0 (Control)	24	Static medium large gas phase (60-ml roller bottle)	9	—	2.3	7	2	Not recorded				
	10 min			9	—	2.4	8	1					
	0.5			9	—	2.5	9	0					
	1			9	—	1.9	8	1					
2	0 (Control)	24	Static medium with large gas phase (sealed watchglass)	5	—	2.1	5	0	8				Not relevant to 24-h cultures
	24			5	—	2.2	5	0	9				
2, 4, 6	0 (Control)	24	Static medium with small gas phase (<0.1 ml) (sealed embryological watchglass)	5	—	1.2	4	1	7				
	2			7	—	1.5	4	3	●				
	4			7	—	1.1	0	7	●				
	6			7	—	0.9	0	7	●				
1, 2	0 (Control)	48–50	Flowing medium with small gas phase (0.5 ml) for first 24 h (sealed Falcon plastic test tube)	20	187 ± 10	4.0	20	0	23	19	1	18	2
	24			20	147 ± 7	3.5	20†	0	22	18	2	14	6
	2			4	64 ± 4	2.9	4‡	0	11	3	1	0	4

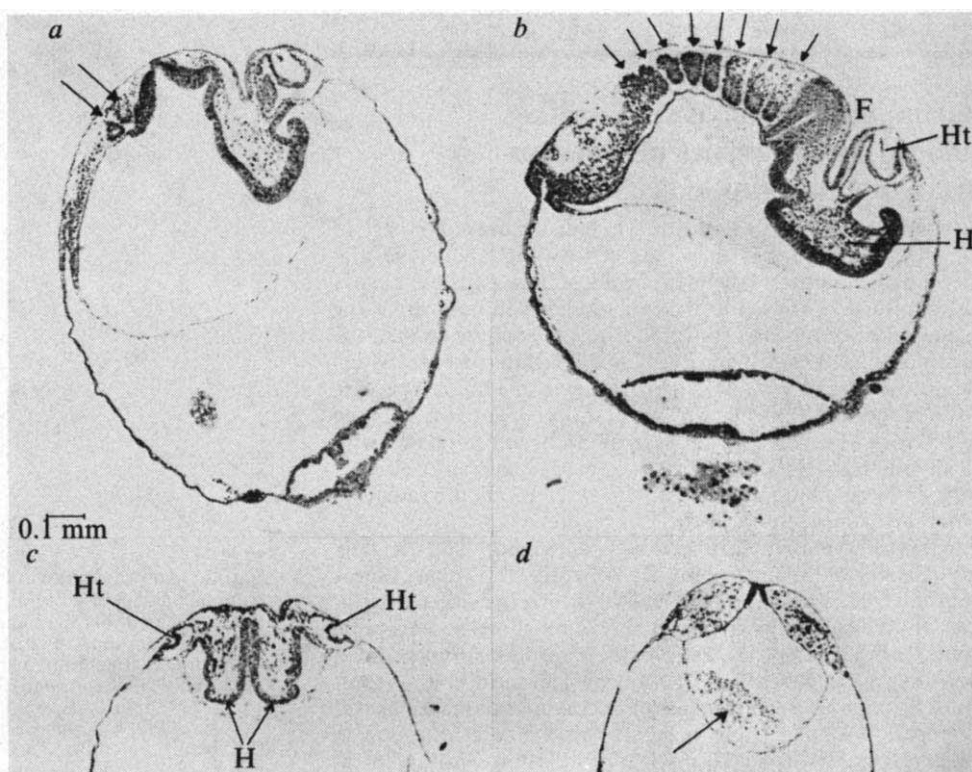
●, Indistinguishable.

*5% CO₂ in air for total culture period.

†One out of twenty overexpanded pericardia.

‡Two out of four overexpanded pericardia.

Fig. 1 *a*, Near sagittal section of conceptus cultured for 24 h in twice the standard dose of Avertin. Two poorly formed somites can be seen (arrowed). *b*, Sagittal section of control conceptus cultured for 24 h. Note the larger number of well formed somites (arrowed) compared with (*a*). *c* and *d*, Frontal sections of conceptuses cultured for 24 h in twice the standard dose of Avertin. The unfused heart primordia on either side of the head is shown in (*c*), and the cellular debris (arrowed) found in the amniotic cavity of experimental embryos is shown in (*d*). The scale is same for all figures: bar represents 0.1 mm; F, foregut; H, head; Ht, heart.



dose of Avertin. As in twice the standard dose, all embryos cultured for 48 h developed a heartbeat and closure of the neural folds was unaffected. The frequency of embryos which had 'turned', however, was decreased in the standard dose and twice the standard dose of Avertin compared with the controls. Differentiation, assessed by somite number, was unaffected by the standard dose, but was greatly retarded in twice the standard dose.

Histological examination of control embryos and those cultured in twice the standard dose of Avertin confirmed the detrimental effect of the anaesthetic on differentiation. In the experimental embryos the somites were poorly differentiated and fewer in number (Fig. 1*a*) compared with control embryos (Fig. 1*b*). As well as this effect on the paraxial mesoderm the heart seemed to be affected (Fig. 1*c*). In four out of seven embryos cultured in twice the standard dose of Avertin, cell debris was present in the amniotic cavity (Fig. 1*d*). This was also found when embryos were cultured in four and six times the standard dose. In the latter group the embryonic material consisted almost entirely of dead or dying cells. Debris was not found in the amniotic cavity of control embryos.

These experiments have demonstrated that the anaesthetic Avertin can be teratogenic in the rat, and that the degree of embryotoxicity is dose dependent. When a range of parameters of development was examined, rat embryos which had been cultured in serum containing Avertin were retarded compared with controls. It is not clear whether the type of growth retardation which was observed in apparently normally differentiating embryos as a result of treatment with standard dose of Avertin could be corrected by compensatory growth later in gestation¹⁵.

The main advantage of the model used here over, for example, cell lines in tissue culture as a teratological test system, is that the use of whole embryos more closely resembles the situation *in vivo*. There is, however, the important difference that all the observations reported here result from the direct effect of known doses of Avertin on the embryo, independent of secondary maternal factors such

as placental permeability. Further, the anaesthetic is metabolised *in vivo*, and the metabolic products may be more or less teratogenic than the original agent.

In view of these positive results, the factors which remain to be examined include the establishment of the minimal teratogenic dose and exposure duration of a range of anaesthetics. Experiments should also be carried out with embryos of different gestational ages, and different species. It would also be interesting to compare the sensitiveness of our experimental model with other models now in use for teratological testing.

It seems likely that anaesthetics other than Avertin may be teratogenic in species other than the rat. It would therefore be of clinical importance to determine whether any of the anaesthetic agents commonly used in human practice have a similar embryotoxic action when administered during early pregnancy.

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Partial chromosomal set labelling induced by ultraviolet microbeam irradiation of mitotic cells

It would be useful to find out whether a particular part of the chromosome set in the living cell can be labelled so that the labelled and unlabelled chromosomes can be clearly distinguished. This would make it possible to determine the localisation of chromosomes during interphase when the chromosomes are dispersed as tangled thin threads which cannot be traced with the light or electron microscope. We have suggested¹ that an ultraviolet microbeam can be used to provoke the labelling of part of a chromosome set with ³H-thymidine (³H-TdR). Here we present data on mammalian cell labelling following ultraviolet microirradiation which are consistent with this suggestion.

When mammalian cells are incubated with ³H-TdR only the cells normally undergoing DNA synthesis (S phase) show uptake of the tracer into the DNA. After ultraviolet irradiation the cells not in S phase may also show a detectable uptake of ³H-TdR (ref. 2). This is a result of unscheduled DNA synthesis which is generally considered to be the result of excision and replacement of ultraviolet-damaged bases by repair enzymes³. The unscheduled DNA synthesis also takes place after a partial nuclear irradiation with an ultraviolet microbeam. Ultraviolet microirradiation produces an alteration in an irradiated volume of nucleoplasm without apparently affecting the remainder of the nucleus or the surrounding cytoplasm⁴. It was found that ultraviolet microirradiation of the nucleus of HeLa or KB cells not in S phase produced a localised ³H-TdR incorporation only over the zone actually irradiated by ultraviolet light^{4–6}. Such observations of partial nuclear labelling have been obtained in experiments with interphase cells. During mitosis the chromosomes condense into clearly defined thick strands and the microbeam can be focused on to a distinct part of the chromosome set or even to a small region of one chromosome⁷. We have proposed that DNA of the microirradiated mitotic chromosomes can also be selectively labelled with ³H-TdR and the postmitotic localisation of the labelled DNA can be determined by autoradiography.

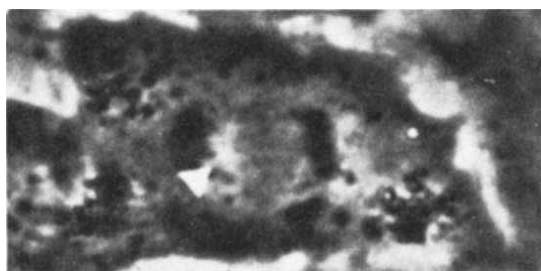


Fig. 1 Typical anaphase SPEV cell during ultraviolet microirradiation. The light triangle over the left chromosomal group indicates the area of irradiation (exposure 5 s at a dose rate of $\sim 2 \times 10^{-3}$ erg μm^{-2} s⁻¹). Phase contrast $\times 1,500$.

The experiments were carried out on pig embryo kidney cells (SPEV) routinely maintained in medium 199 supplemented with 10% foetal calf serum. The cells were used 24 h after they were seeded in monolayer on a quartz coverslip mounted in a window of a sterile culture chamber. These cells were chosen because they remain relatively flat during the mitotic process, thus making the chromosome set easy to irradiate. The cells were irradiated through the coverslip by an inverted monochromatic (280 nm) ultraviolet microbeam with the spot area about $4 \mu\text{m}^2$. A suitable

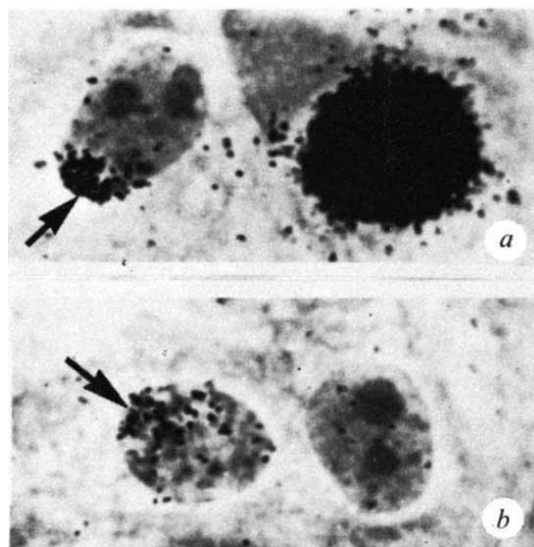


Fig. 2 Autoradiographs of interphase SPEV cells. The cells were incubated in culture medium containing ³H-TdR ($10 \mu\text{Ci ml}^{-1}$). Incubations were carried out for 1.5 h immediately after partial irradiations of anaphase chromosomes. The cells were then rinsed with culture medium and fixed in 3:1 ethanol-acetic acid. After treatment with 3% perchloric acid, autoradiographs using M emulsion were prepared and developed after 10 d exposure. Preparations were stained with haematoxylin. a and b (left hand), Postmitotic nuclei containing a portion of irradiated chromosomes. Arrows indicate the "patches" of unscheduled ³H-TdR labelling. a (right hand), Non-irradiated nucleus in normal S phase. b (right hand), Unlabelled control nucleus not in S phase, $\times 1,500$.

dose was determined empirically by varying the time of irradiation. During metaphase or anaphase 20–25% of the chromosome set of each cell was irradiated. The chromosomal target was always located near the edge of the metaphase plate or within one group of anaphase chromosomes. The pattern of the anaphase cell irradiation is shown in Fig. 1. After irradiation of the anaphase cell only one of the daughter cells obtained a portion of irradiated chromosomes, but the other one could be used as a control. Incorporation of ³H-TdR into postmitotic nuclei was studied by autoradiography.

Fifty-six irradiated cells were studied. Within 1.5–2 h of irradiation these cells had completed cell division and formed daughter nuclei with decondensed chromosomes. These nuclei contained apparently normal interphase nucleoli, but sometimes they also included some pre-nucleolar bodies. The appearance of these bodies may be due to nucleolar organiser damage by ultraviolet microirradiation^{8,9}.

Autoradiography showed that partial ultraviolet irradiation of the mitotic chromosomes provoked unscheduled DNA synthesis in all cases. This synthesis can be clearly detected in the daughter nuclei as patches of unscheduled incorporation of ³H-TdR. These labelled patches are presented in Fig. 2a and b. The label was never seen in the nuclei of the control daughter cells, since all these cells were not in S phase. The average grain number in the labelled patches (28 grains) was about 20 times greater than that in control nuclei. In the 22 nuclei studied the labelled patches occupied no more than 30% of the nucleus area (Fig. 2a). The remaining 34 nuclei exhibited incorporation of ³H-TdR in 30–50% of the nucleus area (as shown in Fig. 2b). It is extremely likely that these patches of labelling are situated over the sites of the postmitotic localisation of the irradiated chromosomes.

Our results provide the first demonstration of localised DNA synthesis in interphase nuclei following ultraviolet microirradiation of mitotic chromosomes. Further studies

are in progress with the aim of using this technique in an attempt to elucidate the localisation of different parts of the chromosome set during interphase. These studies should take into account the possible influence of irradiation on the postmitotic arrangement of chromosomes. Further experiments should, however, be carried out on cells with chromosomes which are few in number and large in size.

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Mutation frequency at a recessive locus in haploid and diploid strains of a slime mould

It is puzzling that mutants are isolated at a similar frequency in tissue cultures of haploid and diploid strains^{1–3}, for twice as many mutations have often been assumed necessary to produce a mutant diploid as to produce a mutant haploid. Explanations for the apparent high frequency of mutation in diploids^{1–6} include epigenetic effects^{1–3}, changes in gene dose⁷, dominant mutations⁸, recessive X-linked mutations⁴ and heterozygosity before selection⁹. Most recessive autosomal mutants are assumed to arise because of haploidy for the chromosome containing the mutant locus, since most cell cultures are aneuploid^{4,10,11}. It is difficult to measure the mutation frequency at recessive loci in cultured haploid and diploid strains because of the paucity of haploid strains and the problems caused by aneuploidy. The slime mould *Dictyostelium discoideum*, a simple eukaryote, is suitable for this purpose, however, as both haploid and diploid strains are available^{12–14} and aneuploids are so unstable or inviable that none has been isolated¹⁴. Using this organism, I have found, contrary to the expectation that mutant diploids should be extremely rare (say 2×10^{-12} diploid, 10^{-6} haploid)^{1–6}, that the difference in mutation frequency between haploids and diploids for one recessive locus is only an order of magnitude.

Isogenic haploid (NP73) and diploid (DP62) strains of *D. discoideum* able to grow in axenic medium and derived from strain V12 (K.L.W., in preparation) were used. Strains NP73 and DP62 were isolated from the same culture, and strain DP62 is a diploid, formed presumably by the parasexual fusion of two haploid amoebae of strain NP73. The fruiting bodies of both strains have a similar appearance when amoebae grown on slime mould (SM) agar plates with *Aerobacter aerogenes* are allowed to sporulate, although growth of DP62 is slightly slower and the spore masses are shorter than NP73. All 28 haploid segregants of strain DP62, obtained by prolonged growth of DP62 in axenic medium¹⁴ or as faster growing sectors on agar plates, were indistinguishable from strain NP73. Both strains carry the

Table 1 Spontaneous frequency of occurrence of methanol resistance (*acrA*) in isogenic haploid and diploid strains of *D. discoideum*

Strain	No. of mutants*	Frequency $\pm$ s.e.
Haploid NP73	115	$7.2 \pm 1.6 \times 10^{-7}$
Diploid DP62	15†	$8.8 \pm 4.6 \times 10^{-8}$

The table shows the combined results from the examination of 1.6×10^8 amoebae of strains NP73 and DP62. For each of eight clones, 10 methanol-containing (2% v/v) SM agar plates¹⁷ were inoculated with 2×10^6 amoebae and a heavy inoculum of the bacterial associate *Aerobacter aerogenes*. We had established that the frequency of occurrence of *acrA* mutants is independent of the number of amoebae plated over a wide range of inoculation densities (at least 10^5 – 2×10^6 per plate)¹⁷. Resistant colonies were isolated from the methanol-containing SM agar plates after 7 d at 22 °C; no additional resistant colonies were observed at day 14. All resistant colonies were passaged on SM agar and retested on SM agar containing methanol to confirm that resistance was maintained in the absence of methanol. The plating efficiency on SM agar containing methanol (2% v/v) of two haploid and two diploid methanol-resistant strains studied further was 100%. The ploidy of all mutants was assessed by spore size and shape. The ploidy of several methanol-resistant haploid and diploid strains was confirmed by chromosome staining (Fig. 1).

*Haploids, *acrA*; diploids, *acrA/acrA*.

†Includes one haploid strain which is excluded in calculating the frequency.

spore shape marker *sprB2* (ref. 17) which makes the distinction between haploid and diploid strains very easy (Fig. 1a and b). To confirm the ploidy, both strains were grown axenically and their chromosomes were stained; NP73 has seven chromosomes and DP62 has 14 (Fig. 1c and d).

The *acrA* locus located on linkage group II was studied because it is the only site of methanol resistance in *D. discoideum*^{15,16}. The phenotype of strains with a mutation at the *acrA* locus is recessive resistance to acriflavin ($100 \mu\text{g ml}^{-1}$), methanol (2% v/v) and thiabendazole ($10 \mu\text{g ml}^{-1}$)¹⁵. Isogenic haploid (NP73) and diploid (DP62) strains were plated on to SM agar plates containing methanol (2% v/v)¹⁵ to obtain mutants at the *acrA* locus.

Table 1 summarises the results of experiments where 2×10^7 amoebae of each of eight different clones of strains NP73 and DP62 were plated on SM agar plates containing 2% methanol. All experiments with haploids and diploids were conducted in identical conditions using the same batches of methanol-containing plates. All resistant mutants shown in Table 1 maintained resistance to methanol when passaged in the absence of methanol. Ploidy was determined according to spore size and shape, and in some cases chromosome numbers (Fig. 1).

Spontaneous mutations to methanol resistance in haploid strain NP73 occurred at a frequency of 7.2×10^{-7} ; this is within the range reported for other haploid strains of *D. discoideum* (10^{-7} – 2×10^{-6} , refs 15–18). Spontaneous methanol-resistant diploids were isolated from strain DP62 at a frequency of 8.8×10^{-8} ; this was approximately eight times less than the frequency in the haploid strain. A single haploid, methanol-resistant strain was obtained from strain DP62 (Table 1). This strain probably arose either by early haploidisation of a methanol-resistant diploid, or by mutation in a haploid amoeba in strain DP62. All diploid strains of *D. discoideum* contain haploids at a low frequency, usually $< 10^{-3}$.

If methanol resistance in the diploids was due to homozygosity for a recessive mutation, all haploid segregants from such a diploid would be resistant to methanol. On the other hand, if resistance arose due to a dominant mutation, only half of the haploid segregants should be resistant to methanol. To rule out the possibility of dominant alleles at the *acrA* locus, 15 independently derived haploid segregants isolated as faster growing sectors from a methanol-resistant diploid on SM agar plates were screened for methanol resistance. All were resistant, confirming observations that mutations at the *acrA* locus are recessive^{15–18}.

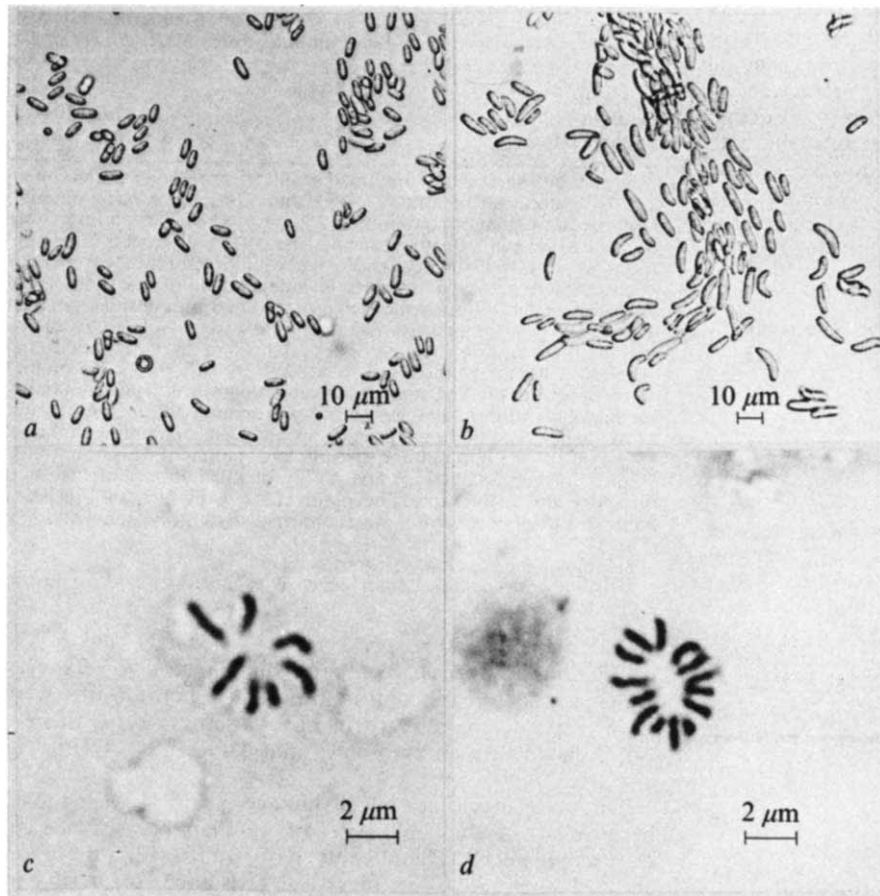


Fig. 1 *a*, Spores of haploid strain NP73 ($\times 300$); *b*, Spores of diploid strain DP62 ($\times 300$); the diploid spores are slightly boomerang-shaped and are considerably longer than the haploids. *c*, Late prophase of haploid strain NP73 showing seven chromosomes ($\times 3,250$). *d*, Late prophase of diploid strain DP62 showing 14 chromosomes ($\times 3,250$). Both strains were grown axenically and the chromosomes were fixed and stained with Giemsa stain as described previously¹⁴.

Studies of diploid seeds of the tomato *Lycopersicon esculentum* have shown that, when the mutagen hydrazine is used, the frequency of mutants homozygous at specific recessive loci is similar to that of mutants heterozygous at the same loci¹⁹. Together with these results, my studies of *D. discoideum* show that the unexpectedly high frequency of mutants homozygous at recessive loci in diploids is not a unique property of cell tissue culture systems; it may even be a general property of diploid cells. An attractive hypothesis which may explain the observed high frequency of mutants at recessive loci in diploids is that they arise by a single mutation followed by recombination, for example, mitotic crossing over or gene conversion, rather than by two independent mutagenic events^{8,11}.

Tests of this hypothesis are in progress, using a methanol-sensitive diploid strain of *D. discoideum* with markers flanking the *acrA* locus—linkage group II, centromere, *whiA*/+, +/+, *tsgD*/+ (ref. 17). The recessive, growth-temperature-sensitive marker *tsgD* is further from the centromere than *acrA*. If methanol-resistant diploids arise by a single mutation followed by mitotic crossing over, then 50% of methanol-resistant diploids would be temperature sensitive for growth, *tsgD/tsgD*, provided that there is an equal probability of either homologue being mutated. Examination of 20 independently derived methanol-resistant diploids provided some support for this hypothesis, for six were growth-temperature-sensitive and 14 grew at the restrictive temperature. There are however, some problems. The high mutation frequency ($\sim 10^{-7}$) does not merely result from sequential mutation and mitotic crossing over without interaction between these processes, since the expected frequency on this basis would be approximately 10^{-10} , that is $\sim 10^{-6}$ (mutation frequency, refs 15 and 17) $\times 10^{-4}$ (frequency of mitotic crossing over between the centromere and *acrA*)^{15,17}.

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Metabolic cooperation between human fibroblasts with normal and with mutant superactive phosphoribosylpyrophosphate synthetase

METABOLIC cooperation is a form of intercellular communication by which cells in contact exchange molecules, a process providing multicellular organisms with an important mechanism for control of metabolic activity¹. Subak-Sharpe *et al.*² observed

contact-dependent transfer of purine nucleotides from normal cells to cells mutationally incapable of producing inosinic acid due to deficiency in hypoxanthine-guanine phosphoribosyl-transferase³. Metabolic cooperation of this type was later also demonstrated with other enzymic markers, such as adenine phosphoribosyltransferase and thymidine kinase^{4,5}. Such transfers are characterised by the normal cell being the donor and the mutant cell being the recipient, the former transferring to the latter a mutationally lacking metabolite. We report here on a new form of contact-dependent metabolic cooperation, unique in that the transfer of a metabolite occurs from a mutant donor cell to a normal recipient cell.

We were able to demonstrate this phenomenon for cultured human fibroblasts using a new enzymic marker, a mutant feedback-resistant superactive phosphoribosylpyrophosphate (PRPP) synthetase. The principle of transfer along a gradient is, however, maintained in that the donor mutant cell is metabolically more active than the recipient normal cell. This marker has been found to be the primary abnormality underlying excessive purine production in a family affected with primary metabolic gout^{6,7}. The mutation is manifest in superactivity of the enzyme in the physiological cellular milieu, being due to decreased sensitivity of the mutant enzyme to feedback inhibition by several physiological intracellular inhibitors such as ADP, GDP and 2',3-diphosphoglyceric acid. This superactivity increases availability of its reaction product PRPP, a key substrate for both *de novo* and salvage pathways of purine nucleotide synthesis. The consequent increased salvage capacity thus enables selection between normal and mutant cells, and the excessive *de novo* purine synthesis enables determination of the proportion of mutant cells in a culture containing a mixture of mutant and normal cells.

The fibroblast growth medium was modified⁸ to allow survival of only the mutant cells, that is, cells possessing increased capacity to produce purine nucleotides. 6-Methylmercaptapurine riboside (6-MMP-riboside; 0.2 mM) was added to block *de novo* purine synthesis⁹, and hypoxanthine (0.2 mM) and uridine (0.5 mM) were added to enable salvage synthesis of inosinic acid and uridylic acid, respectively. Experiments were designed to determine whether the capacity to resist selection in these conditions could be transferred from mutant to normal cells (for details, see Table 1). Normal and

mutant cells were mixed in ratios of 1:1 and 1:3, respectively, and cocultured for 3–10 generations. Table 1 shows that the capacity of mutant cells to resist selection could be transferred to the normal cells, the degree of transfer depending on the degree of contact between normal and mutant cells during selection—the greater the proportion of mutant cells in the cell mixture and the greater the density of the cells in culture during selection, the greater the probability of contact between the mutant and normal cells. After exposure to selection of a normal-mutant cell mixture (1:1) at high cell density, the rate of *de novo* purine synthesis was similar to that found before selection, being the average of the synthesis rates of separate normal and mutant cell cultures. On the other hand, when the same mixed cultures (1:1) were exposed to selection at low cell density, the rate of purine synthesis after selection increased to approximately twofold, indicating survival of the mutant cells only. The acquired resistance of the normal cells against selection was markedly reduced when normal cells were mixed with mutant ones at a 3:1 ratio. After exposure to selection of such a cell mixture at low cell density, the surviving cells were too few to allow propagation to confluency, indicating the death of most of the normal cells. When the same 3:1 cultures were exposed to selection at high cell density, however, there were enough survivors to allow propagation, and the rate of *de novo* purine synthesis in the culture was increased to 2.5-fold compared with the preselection value. These results indicate that in this latter experiment a small proportion of the normal cells survived the selective conditions, because, if all normal cells have been killed, the rate of purine synthesis should have increased approximately to fourfold.

We are assuming that the increase in the rate of purine synthesis reflected death of the normal cells. That this was indeed the case, and that there was no increase in the rate of *de novo* purine synthesis in mutant cells, is evident from experiments in which mutant cell cultures were similarly exposed to the selective conditions. In such mutant cells the rates of purine synthesis before and after selection were found to be the same.

That this metabolic cooperation was contact dependent was determined as follows. One coverglass covered with normal fibroblasts and two coverglasses covered with mutant fibroblasts were placed side by side without physical contact

Table 1 Rate of *de novo* purine synthesis in mixed normal-mutant fibroblast cultures after selection against normal cells

Source of cells	Rate of incorporation of ¹⁴ C-formate into purines excreted by cells into incubation medium (c.p.m. per mg protein per 6 h)		
	Before selection	After selection using:	
		Dense cultures	Dilute cultures
Normal subjects			
J.B.	8,120	All cells killed	
J.Be	12,050	All cells killed	
Subject O.G. with mutant enzyme	89,293	106,301	86,668
Mixture of normal and mutant cells			
O.G. + J.B. 1:1	59,294	70,447	94,567
O.G. + J.Be 1:1	58,717	61,163	90,116
O.G. + J.Be 1:3	27,269	66,246	Too few survivors to allow propagation

Biopsies (3 mm) were removed from the forearm skin, minced, placed in Falcon Plastics sterile disposable bottles and flooded with Eagle's MEM containing Earle's Balanced Salt Solution (Gibco, F-15), 15% foetal calf serum, penicillin (100 U ml⁻¹), streptomycin (100 µg ml⁻¹) and mycostatin (25 µg ml⁻¹). Media were saturated with a 5% CO₂-95% air environment, and the bottles stoppered and incubated at 37 °C until outgrowth was apparent. Cells were subcultured and serially propagated until confluency. Cell mixtures which were prepared after trypsinisation, were cocultured for 2–3 generations. Homogeneous and mixed cultures were exposed to selection at high (25,000 cells cm⁻²) and low (1,000 cells cm⁻²) cell densities, by addition to the regular culture medium of 0.2 mM 6-MMP-riboside, 0.2 mM hypoxanthine and 0.5 mM uridine. The selection medium and regular medium were changed every second day. Selection was carried out for one week, after which the cultures were flooded with the regular medium and propagated until confluency. Cells were collected by trypsinisation and divided into two Falcon flasks and propagated until half confluency was obtained (0.5–1.5 × 10⁶ cells per 75-cm² Falcon Plastics culture flask), at which stage the cultures were studied for the rate of *de novo* purine synthesis by measuring the incorporation of ¹⁴C-formate into total purines excreted by the intact cells into the incubation medium, essentially as described previously^{7,8}. The non-confluent monolayer cultures were incubated for 6 h with 10 ml fresh growth medium containing 10 µCi ¹⁴C-formate. At the end of incubation, the total volume of the incubation medium, after removal of cells by centrifugation, was transferred to tubes and acidified with 2.0 ml 6 N perchloric acid; 0.2 µCi 8-³H-adenine were added and the tubes thoroughly mixed and maintained at 100 °C for 1 h. The hydrolysate was centrifuged and the supernatant adjusted with water to 15 ml and chilled. To each tube 7 mg adenine was added as carrier, the purine bases precipitated as purine silver complex, washed, extracted with 0.1 N HCl at 100 °C, and the radioactivity was counted. Results were corrected for incomplete recovery of purines, using 8-³H-adenine as reference, and related to total cell protein. For all fibroblasts, labelling of purines excreted into the medium was linear with time for at least 8 h, and, for a fixed period of incubation, was also proportional to the number of cells incubated.

in one Petri dish, and flooded with selective medium. The normal cells started to degenerate within 24 h, all dying within 1 week, whereas the mutant cells remained alive without signs of degeneration.

No attempt has been made as yet to identify the molecule, the contact-dependent transfer of which is responsible for the metabolic cooperation. Four possibilities may be considered: transfer of inosinic acid or other purine nucleotides, transfer of the enzyme PRPP synthetase or of its reaction product PRPP, or transfer of molecules containing the information for the synthesis of the mutant superactive PRPP synthetase, such as DNA or RNA. That the transferable molecule has a short lifespan in the recipient cell is indicated by the dependence of metabolic cooperation on the presence, during selection, of close contact between the mutant and the normal cells, and by the observation that prolonged contact between normal and mutant cells before exposure to selective medium does not render normal cells resistant to selection. In view of what is known about contact-dependent metabolic cooperation¹⁻⁵, the short lifespan may indicate that this molecule is either the reaction product, PRPP, or purine nucleotides. That nucleotides are transferred has already been demonstrated in other cases of contact-dependent metabolic cooperation; whether PRPP is also transferred has not yet been clarified. Nevertheless, a transfer of PRPP is likely in view of its higher concentration in the donor cell and its small molecular weight.

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Altered glucose-6-phosphate dehydrogenase in bromodeoxyuridine-substituted cells

It has been adequately demonstrated that BUdR is mutagenic in bacteriophage^{1,2,3} when incorporated in place of thymidine in DNA, whereas a clear demonstration that it is mutagenic in eukaryotic cells is still lacking. We have described the selection of two variant hamster cell lines that survive with extensive levels of BUdR substitution in DNA^{4,5} and that have been maintained in continuous cultivation for more than 300 generations without loss of viability. We have found no evidence of alterations in the nucleotide ratio of nuclear DNA consistent with the accumulation of BUdR-induced base transition on long term cultivation of the cells, and analysis of rRNA made in the substituted cells offers no evidence that BUdR causes base transitions within the genome or transcriptional errors which result in the accumulation of faulty RNA transcripts⁶.

As a more sensitive test to detect BUdR-induced alterations in the genome or in transcription products, we have begun to examine various enzymes produced in the parent and substituted cell lines. The heat lability of an enzyme is frequently used as one criterion of an altered primary sequence of that protein. Thus, senescent WI-38 and MRC-5 cell cultures have increased fractions of heat labile glucose-6-phosphate dehydrogenase (G6PD)⁷ and lactic dehydrogenase⁸; ageing mice have an increased number of inactive liver aldolase molecules⁹, and cultured fibroblasts taken from patients with either progeria¹⁰ or Werner's

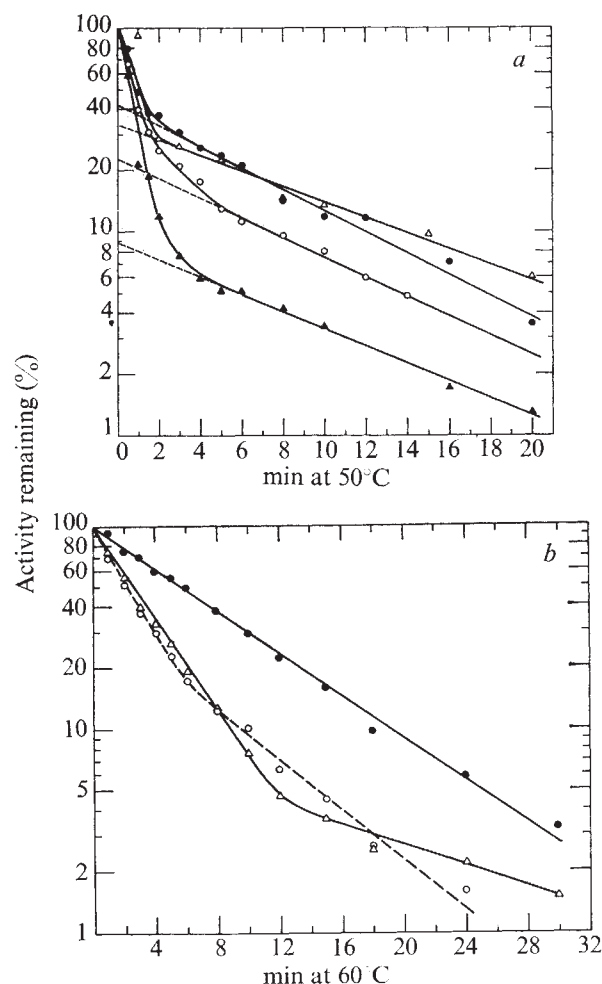


Fig. 1 *a*, Heat inactivation of G6PD from BUdR-substituted cells. Confluent roller bottles were rinsed twice with 250 mM sucrose, 1 mM EDTA, and 1 mM 2-mercaptoethanol, pH 8.2, and the cells were scraped off in the same solution. Subsequent steps were carried out at 4 °C. Cells were pelleted at 1,500 r.p.m. for 10 min, resuspended in the sucrose solution at approximately 10^7 cells per ml and sonicated by three bursts of 10 s each at 7 mA in a Bronwill sonicator (microprobe). Debris was removed by centrifugation at 600g for 10 min, and the supernatant then spun at 100,000g for 1 h. Protein was determined by the method of Lowry *et al.*²¹, and all samples were adjusted to approximately 1.5 mg protein per ml and stored in liquid nitrogen. Assays for G6PD activity contained: 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 1 mM NADP and 2 mM G6P. The final volume was 1 ml, containing from 0.025 to 0.2 ml of enzyme. Enzyme samples were heated at 50 °C for the appropriate time, quenched on ice and transferred to the complete reaction mixture at 25 °C. Assays were carried out at 25 °C in a Gifford 2400 spectrophotometer, following the initial rate of increase in absorbance at 340 nm due to the reduction of NADP. Activity in the unheated enzyme was dependent on the addition of both G6P and NADP. ●, 3460; ○, B4; ▲, HAB; △, a mixture of 3460 and HAB with relative G6PD activities of 2:1. *b*, Heat inactivation of G6PD isolated in the presence of 1 mM NADP. Enzyme samples were prepared and assayed as in (a) except that the enzyme was always in the presence of 1 mM NADP and heated at 60 °C. ●, 3460; ○, B4; △, HAB.

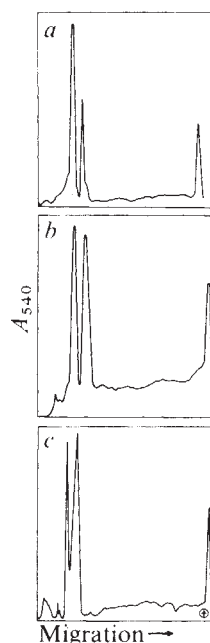


Fig. 2 Electrophoresis of G6PD on polyacrylamide gels. Samples were electrophoresed for 4 h and 3 mA per gel on 11-cm 8% polyacrylamide disk gels according to Bakay and Nyhan²¹. The running buffer was 5 mM Tris-HCl, pH 8.1, and 40 mM glycine. Bromophenol blue was added as a marker and ran to about 1 cm from the end of the gel at the termination of electrophoresis. Gels were stained for G6PD activity for 45 min at 37 °C in a solution containing: 100 mM Tris-HCl, pH 7.6, 10 mM MgCl₂, 0.5 mM NADP, 3.5 mM G6PD, 0.73 mM nitroblue tetrazolium, and 0.2 mM phenazine methosulphate. Gels were then fixed in 7% acetic acid and scanned at 1 cm per min for G6PD activity at 540 nm in a Gilford 2400 spectrophotometer fitted with a linear transport unit. Electrophoresis is from left to right, with the bromophenol blue marker at the extreme right. Peaks of G6PD activity are designated I and II corresponding to the most slowly and rapidly migrating, respectively. *a*, 3460; *b*, B4; *c*, HAB.

syndrome^{11,12}, both of which show clinical manifestations of premature ageing, have an increased fraction of heat labile G6PD, 6-phosphogluconate dehydrogenase and hypoxanthine-guanine phosphoribosyl transferase.

If BUdR is acting as a mutagen in the substituted hamster cell lines, we might expect to find differences in the heat lability and/or other enzymatic properties of some enzymes which could be the consequence of alterations in the primary sequence of proteins due to BUdR-induced base transitions during replication or transcription. We report here that both *in vitro* and *in vivo* experiments have shown that substituted cells contain an altered population of G6PD molecules, which correlates well with the level of BUdR substitution in the genome.

The cell lines used have been described before^{4,5}. Briefly, 3460 cells are derived from a Syrian hamster melanoma (RPMI 3460), are grown in Dulbecco's modified Eagle's medium in the absence of BUdR and routinely have a cloning efficiency of >95%. B4 cells are derived from 3460, are grown in the presence of 10⁻⁴ M BUdR (required for optimum growth) and have a cloning efficiency of ~90%;

the nuclear DNA is 62% substituted with BUdR. HAB cells are derived from B4 by growth in media containing 0.1 mM hypoxanthine, 0.4 μM aminopterin, and 10⁻⁵ M BUdR; the cloning efficiency is routinely ~85% and the nuclear DNA is ≥ 99.8% substituted with BUdR. The generation times are approximately 16, 28 and 36 h for 3460, B4 and HAB cells, respectively. Both B4 and HAB cells have undergone more than 300 population doublings without apparent loss of viability and had undergone more than 175 population doublings at the time of our experiments.

Human erythrocyte G6PD consists of a single polypeptide chain encoded for by an X-linked gene¹³, and is active in both dimeric and tetrameric forms, but inactive as a monomer¹⁴. This is probably true of rodent cells as well¹⁵, although the enzyme activity may be partially regulated by autosomal loci¹⁶. For heat denaturation studies crude enzyme preparations were made from 3460, B4 and HAB cells (in the absence of NADP). Their heat lability is shown in Fig. 1*a*. The G6PD activity from all three cell lines shows a biphasic denaturation, with both a rapidly and a slowly denaturing component. When extrapolating the linear portion of the curve (slowly denaturing) to the ordinate, we calculate that the fraction of heat labile G6PD molecules in 3460, B4 and HAB cells is 59%, 77% and 91%, respectively. The average fraction of heat labile G6PD molecules from four different preparations is 56.5%, 69.7%, and 79.7% for 3460, B4 and HAB cells, respectively (Table 1). This difference is not due to different protein concentrations of heated enzyme preparations since they were adjusted to nearly equal protein concentrations before heating and assay. More important, dilutions of each enzyme preparation gave the same value for the fraction of heat labile molecules on extrapolation, although the rate of decay varied with protein concentration (data not shown). It is unlikely that endogenous inhibitors or activators of G6PD activity could account for these differences since a mixture of 3460 and HAB enzyme extrapolates intermediate to the two assayed individually (Fig. 1*a*). This enzyme mixture contained a G6PD activity ratio of 2:1 (3460:HAB), expected to yield, therefore, a value of 69% heat labile (using the value of 59% and 91% heat labile G6PD for 3460 and HAB cells, respectively). The extrapolation was to 66% heat labile. As human G6PD is stabilised in the presence of NADP¹⁴, we have examined the ability of NADP to stabilise G6PD against heat denaturation. When 3460 enzyme was incubated with ≥ 10 μM NADP for 20 min at 4 °C before heat denaturation, we detected no inactivation of G6PD at 50 °C for up to 30 min, whereas ≥ 50 μM NADP is required to stabilise fully B4 and HAB cell G6PD at this temperature (in the presence of 25 μM NADP, HAB enzyme was 25% denatured within 20 min). In as much as NADP is shown to stabilise all three enzyme preparations to some extent, it was possible that heat lability differences were due to different intracellular amounts of NADP and, thus, differential inactivation during isolation. Therefore, enzyme preparations were isolated in the presence of 1 mM NADP and heat inactivation studies carried out at 60 °C. These results are shown in Fig. 1*b*, from which we calculate the fraction

Table 1 Properties of G6PD from BUdR-substituted cells

Enzyme (cell line)	% Heat labile*	NADP (μM)	K _m †	G6P (μM)	dG6P utilisation‡
3460	59,56,58,53 (56.5)	14.5 ± 1		33 ± 2	7.5 ± 0.2
B4	77,66,69,67 (69.7)	20.7 ± 1		33 ± 2	8.9 ± 0.2
HAB	91,74,78,76 (79.7)	23.0 ± 1		33 ± 2	10.7 ± 0.3

*Values are from four different enzyme preparations and their mean (shown in parentheses).

†K_ms were determined after dialysis of the enzyme preparations (isolated in the absence of NADP) against 250 mM sucrose, 1 mM EDTA and 1 mM 2-mercaptoethanol for 16 h at 4 °C. NADP was assayed from 1 to 200 μM and G6P from 5 to 300 μM, in triplicate (± s.d.).

‡Percentage of glucose-6-phosphate utilisation (determined in triplicate from two enzyme preparations, ± s.d.), after dialysis for 16 h.

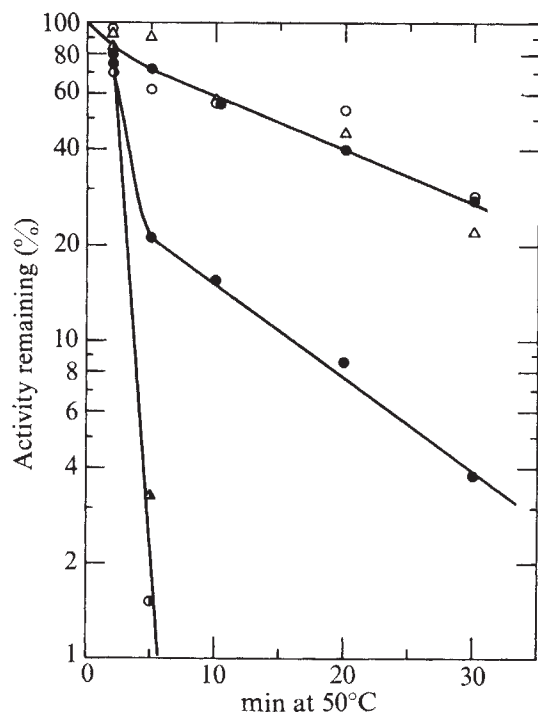


Fig. 3 Heat inactivation of G6PD activities I and II. Enzyme samples (isolated in the absence of NADP) were heated at 50 °C for various times as described in Fig. 1a and then subjected to electrophoresis as described in Fig. 2. Gels were stained for G6PD activity for 45 min at 37 °C, fixed in 7% acetic acid, and then scanned at 540 nm to measure the rate of loss of activities I and II as a function of heating time. All values are plotted as the % activity in the respective peaks relative to unheated controls (the average of quadruplicate gels). ●, 3460; ○, B4; △, HAB. Top curve, peak I; lower two curves, peak II.

of heat-labile G6PD in B4 and HAB cells to be 62% and 91%, respectively. In these conditions, 3460 enzyme decayed linearly.

Criteria used to characterise the large number of human G6PD variants¹⁷ include altered *pH* optima, altered K_m for NADP and glucose-6-phosphate (G6P), and the utilisation of 2-deoxy-D-glucose 6-phosphate (dG6P), an analogue of G6P. Table 1 shows that all three cell lines had the same K_m for G6P, but differed in their K_m for NADP. Furthermore, B4 and HAB cell G6PD had a slightly greater ability to utilise dG6P as a substrate. All three enzymes showed *pH* optima at 7.5 and 8.5 with only minor differences at the extremes of the curves (*pH* 7.0 and 10.0), not unlike many of the human variants¹⁷.

We could not detect different electrophoretic mobilities of G6PD from the three cell lines on cellulose acetate (frequently used to detect human variants) and have used instead polyacrylamide disk gels, where we have been better able to quantitate enzyme activity on the gel (Fig. 2). Two major peaks of activity were observed for all three cell lines with R_f of 0.22 (peak 1) and 0.28 (peak 2), although the relative proportions of peaks 1 and 2 differed significantly. Thus, the relative amounts of peaks 1 and 2 were routinely found to be 69:31, 52:48, and 28:72 for 3460, B4 and HAB cell G6PD, respectively. (These values varied by $\pm 5\%$ on duplicate gels and with different preparations.)

The increasing proportion of peak 2 in B4 and HAB cell G6PD suggests the possibility that this activity is largely responsible for the increasing fraction of heat labile G6PD in BUdR substituted cells. This possibility was tested by heating the enzyme samples at 50 °C for various times, electrophoresing the enzyme, and determining the fraction of activity remaining in each of the two activity peaks (Fig. 3). Peak 1 decayed at approximately the same rate for all three cell lines. Peak 2 from 3460 cells decayed

at a somewhat greater rate than peak 1, whereas peak 2 from B4 and HAB cells decayed substantially faster. Only negligible activity remained after 5 min at 50 °C, making quantitation of activity on the gels difficult.

We have sought to confirm the presence of an altered population of G6PD molecules in B4 and HAB cells using the histochemical staining method described by Wajntal and DeMars¹⁸. In particular, we have used this assay to determine if a higher proportion of B4 and HAB cells in monolayer can be shown to utilise the substrate analogue dG6P. After growth in monolayer for 24 h, all three cell lines were stained for G6PD activity using G6P or dG6P, or in the absence of substrate. Cells were scored as being stained or unstained at both low magnification and high magnification. This analysis provides an absolute number for cells staining positive under high magnification (where cells with only a few blue granules of formazan are readily discernable), as well as information on the relative intensity of staining (as the ratio of cells staining positive at high magnification/low magnification).

The percentage of cells stained positive for G6PD activity in the presence of both substrates is given in Table 2. Under low magnification, an equivalent number of cells stained positive ($\sim 76\%$) in the presence of G6PD for all three lines, whereas about twice as many B4 and HAB cells were positive in the presence of dG6P compared with 3460. Fewer than 1.0% of the cells were positive in the absence of substrate. Since the scoring of cells at low magnification is somewhat subjective, a better analysis of positive cells was obtained under high magnification. In these conditions the percentage of positive cells in the absence of substrate increased, and there were significant differences between the substituted and unsubstituted cell lines, with 14% of the HAB cells scored positive compared with 5.9% of 3460 cells. This may reflect higher endogenous levels of G6P in the slower growing B4 and HAB cells. Nevertheless, the number of positive cells in the presence of either G6P or dG6P increased under high magnification and when corrected for the difference in background staining (minus substrate), all three cell lines had an equivalent number of cells stained positive in the presence of G6P. After correction for background staining, however, B4 and HAB cells had a substantially higher propor-

Table 2 Percentage of cells positive for G6PD activity

Substrate	Cell line		
	3460	B4	HAB
Low magnification ($\times 125$)			
G6P	73.3 (1,943)	76.6 (1,919)	78.5 (1,867)
dG6P	11.1 (1,473)	21.1 (1,325)	22.5 (1,146)
None	<1.0 (937)	<1.0 (889)	<1.0 (1,042)
High magnification ($\times 500$)			
G6P	86.3 (1,212)	92.4 (956)	93.9 (872)
dG6P	26.7 (1,077)	62.4 (996)	69.0 (919)
None	5.9 (702)	12.9 (726)	14.0 (714)
Corrected*			
G6P	80.3	79.5	79.9
dG6P	20.7	49.5	55.0
High magnification/low magnification ratio			
G6P	1.09	1.04	1.02
dG6P	1.86	2.35	2.44

Cells in monolayer were stained for G6PD activity essentially as described by Wajntal and DeMars¹⁸. Flaskettes (Lab-Tek) were inoculated with 10^5 cells which were grown for 24 h. Media was then removed and each Flaskette was rinsed with 5 ml of 0.9% NaCl. Staining solution (2.5 ml) was added and the Flaskettes were incubated for 1.5 h at 37 °C. The staining solution consisted of: 0.1 M Tris *pH* 7.8, 0.5 mM $MgCl_2$, 0.2 mM NADP, nitroblue tetrazolium (0.5 mg ml⁻¹), phenazine methosulphate (0.02 mg ml⁻¹) and 6 mM G6P or dG6P (or no substrate). The plastic cover was removed from the Flaskette and the slide was rinsed in distilled water and counterstained in alcoholic eosin. Cells were scored for blue deposits of formazan at either $\times 125$ or $\times 500$. Numbers of cells scored are given in parentheses.

*Percentage of cells scored positive in the absence of substrate at high magnification subtracted.

tion of cells ($\times \sim 2.5$) stained positive with dG6P as substrate when compared with 3460 cells. Using the ratio of positive cells at high magnification/low magnification, all three cell lines stained similarly in the presence of G6P (ratio ~ 1.1), whereas in the presence of dG6P this ratio increased in the B4 and HAB cells, suggesting an increasing fraction of lightly stained cells in the BUdR substituted populations. This consistency in the ratio of high magnification/low magnification in the presence of G6P for all three cell lines suggests that this difference was not due to higher endogenous G6PD levels in the B4 and HAB cells.

Thus, B4 and HAB cells contain a greater fraction of heat labile G6PD molecules than the parent, unsubstituted, cell line. When enzymes are isolated in the absence of NADP, the 3460 cells also have a high level of heat labile G6PD. Using 56.5% heat labile G6PD in 3460 cells (Table 1) as a baseline and 79.7% heat labile G6PD in HAB cells as the effect of 100% BUdR substitution, we would predict that if BUdR is randomly generating mutations or transcriptional errors (which would be a function of the level of BUdR substitution), cells which are 62% substituted (B4) should contain a population of G6PD molecules of which 69.7% are heat labile. We found an average of 70.8%. When enzymes are isolated in the presence of NADP, 3460 G6PD shows a linear decay whereas B4 and HAB are still 62% and 91% heat labile, respectively. Using the extreme values of 3460 (0%) and HAB (91%), we would predict 56% heat labile for B4 on the basis of BUdR substitution, not far from the 62% observed.

Perhaps the clearest demonstration of an altered G6PD in B4 and HAB cells is the difference in the relative amounts of peaks 1 and 2 obtained by electrophoresis on polyacrylamide gels, and the substantial difference in the heat lability of peak 2. Bakay and Nyhan¹⁹ separated human G6PD into three bands of activity by polyacrylamide disk gel electrophoresis, with mobilities similar to the two major bands resolved from the cells used here. The bands of human G6PD activity are interconvertible by NADPH-producing substances, suggesting that the bands are conformers with and without bound NADP²⁰. Furthermore, agents which adversely affect the binding of NADP to the enzyme (such as NADPH) promote formation of the more rapidly migrating band. We therefore propose that peak 2 of G6PD activity in the cells examined here represents the enzyme form without bound NADP. When gels were run in the presence of NADPH, the enzymes from all three cell lines migrated as a single band with an R_f of 0.28, similar to peak 2 (unpublished observations). This finding is consistent with the B4 and HAB cell G6PD being deficient in NADP binding.

The fact that B4 and HAB cell G6PD has a higher K_m for NADP and an increasing proportion of peak 2 activity which is extremely heat labile suggests the presence of an altered ("variant") population of G6PD molecules, at least some of which may have altered in their NADP binding by amino acid substitution. The correlation between the fraction of heat labile G6PD and the extent of BUdR substitution suggests that BUdR may be acting as a mutagen, generating a number of variant G6PD molecules in the B4 and HAB cell lines, or alternatively, that these "variants" represent the products of transcriptional errors induced by BUdR. Although we have been unable to detect transcriptional errors by direct nucleotide analysis⁶, the analysis of enzymes as reported here is expected to be a more sensitive method of detecting a low level of base (and hence, amino acid) changes. A mutagenic role for BUdR (including transcriptional errors) is somewhat paradoxical in light of the continued viability of the B4 and HAB cell lines. While it seems to offer the most satisfactory explanation of our results, several other enzymes from these cell lines will have to be examined before this question can be answered conclusively.

We thank Bryan Cullen for technical assistance.

Note added in proof: Recent experiments have ruled out a heritable mutational origin for the faulty G6PD and suggest a transcriptional origin.

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Decreased levels of an inhibitor of prostaglandin E 9-ketoreductase activity in chick dystrophic breast muscle

A WIDE range of membrane anomalies is one of the characteristics of dystrophic muscle^{1,2}. Since prostaglandins (PGs) are known to interact with such membrane constituents as adenyl and guanyl cyclase; Mg^{2+} -dependent, Na^+/K^+ -activated ATPase; Mg^{2+} -dependent, Ca^{2+} -activated ATPase; adenylate kinase; and phosphodiesterase³, it seemed desirable to search for a possible relationship between PGs and the dystrophic condition. The activities of two potentially regulatory PG-metabolising enzymes were measured. NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase activities⁴ in the breast muscles of 10-d post-hatching chicks with hereditary muscular dystrophy (New Hampshire strain) and the breast muscles of 10-d post-hatching normal (white leghorn) chicks were the same as those in the leg muscles of these animals. PGE 9-ketoreductase activity^{5,6} in the dystrophic breast muscle was considerably more active than its control (normal) breast muscle. Subsequently, it was found that the normal breast muscle contains a much higher level of an inhibitor of PGE 9-ketoreductase activity than dystrophic breast muscle.

The assay system used to measure PGE 9-ketoreductase activities consisted of increments of cytoplasmic fractions of the muscle extracts, 1.0 μ g PGE₂, and 1.0 mM NADPH. Enzymatic generation of PGF_{2 α} was measured by radioimmunoassay with antibodies directed against PGF_{2 α} . When the cytoplasmic fractions were tested for their effect on the activity of exogenous PG 9-ketoreductase, the enzyme purified from chicken heart⁵ was used. This amount of PGE₂ 9-ketoreductase catalysed the generation of 50 ng PGF_{2 α} after incubation for 15 min at 37 °C. In these experimental conditions, the initial velocity of the catalytic reaction of the purified enzyme is being measured. Analyses of these reaction mixtures by thin-layer chromatography confirmed the enzymatic conversion of PGE₂ to PGF_{2 α} . Extracts of the various breast and leg muscles from normal and hereditary dystrophic chicks were made as follows: chicks were decapitated, and the muscles excised and immediately frozen in liquid nitrogen. For extraction, the individual muscles were suspended in 0.092 M phosphate buffer, pH 7.3, 0.1 mM dithiothreitol (1.0 g muscle per 4.0 ml buffer) and homogenised using a Sorval Omni homogeniser at full speed for 1 min at

0 °C. After stopping the mixer for 5 min, the muscle suspension was homogenised again for an additional minute. After centrifugation at 10,000*g* for 20 min, the supernatant fluid was recentrifuged at 100,000*g* for 1 h, and this supernatant fluid used as the cytoplasmic fraction. Analyses of these cytoplasmic fractions for protein showed that the efficiencies of protein extraction from the four muscles were similar.

PGE 9-ketoreductase activities in terms of ng PGF_{2α} generated from 1.0 μg PGE₂ by 50 and 100 μl cytoplasmic fractions from normal and dystrophic breast muscle (10-d post-hatching), and from the leg muscles of these same chicks, are shown in Fig. 1. Values for the dystrophic breast muscle fall into the same range as those for normal leg muscles and symptom-free leg muscles from the dystrophic chicks. Values for normal breast muscles, on the other hand, are clustered around a much lower level. On increasing the amount of muscle extract from 50 to 100 μl (Figs 1*a* and *b*, and 2*a*) added to the reaction mixture, a disproportionately small increase (even a decrease with normal breast muscle) in PGF_{2α} formation was observed, indicating the presence of an inhibitor in these muscle extracts.

To test this possibility, the effect of extracts from the four types of muscle from two chicks on PG 9-ketoreductase purified from chicken heart was studied. The extracts from normal breast muscle (both the 10-d post-hatching chick and an adult normal chicken) depressed the activity of this preparation considerably more than extracts from dystrophic breast muscle and the leg muscles (Fig. 2*b*). Addition of increasing amounts of extract from the normal breast muscles of the young chicks to the purified PGE 9-ketoreductase first increased enzymatic

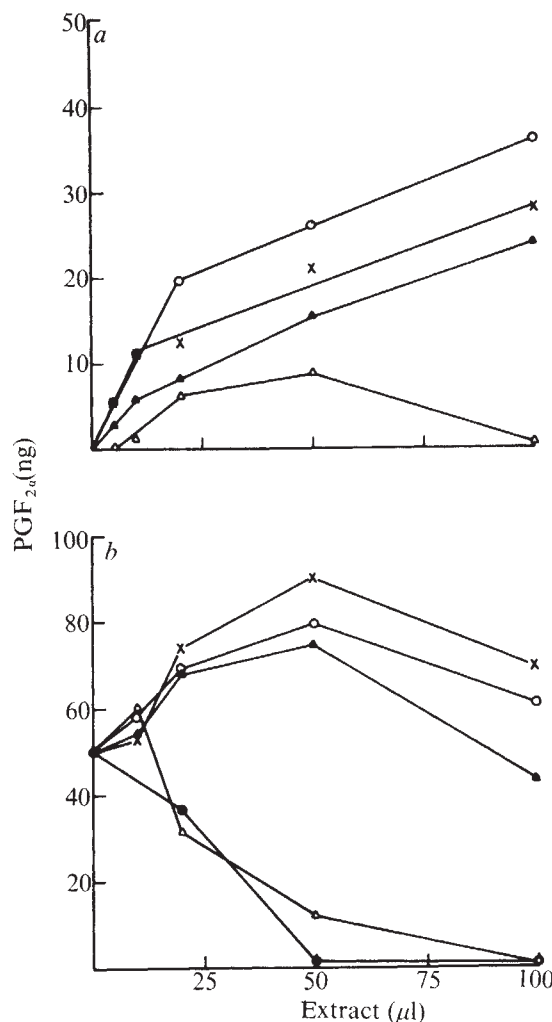


Fig. 1 Enzymatic production of PGF_{2α} by 50 (*a*) and 100 μl (*b*) cytoplasmic fractions of four muscle preparations from individual animals. 1 and 2, Breast muscle: 1, dystrophic chick; 2, normal chick. 3 and 4, Leg muscle: 3, dystrophic chick; 4, normal chick. Enzymatic reaction mixtures, containing the cytoplasmic fractions, 1.0 μg PGE₂ and 1.0 mM NADPH in a total volume of 0.1 ml, were incubated for 15 min at 37 °C. The reaction was stopped by boiling for 2 min. After addition of 0.9 ml buffer, the denatured protein was removed by centrifugation, and PGF_{2α} estimated by radioimmunoassay⁴.

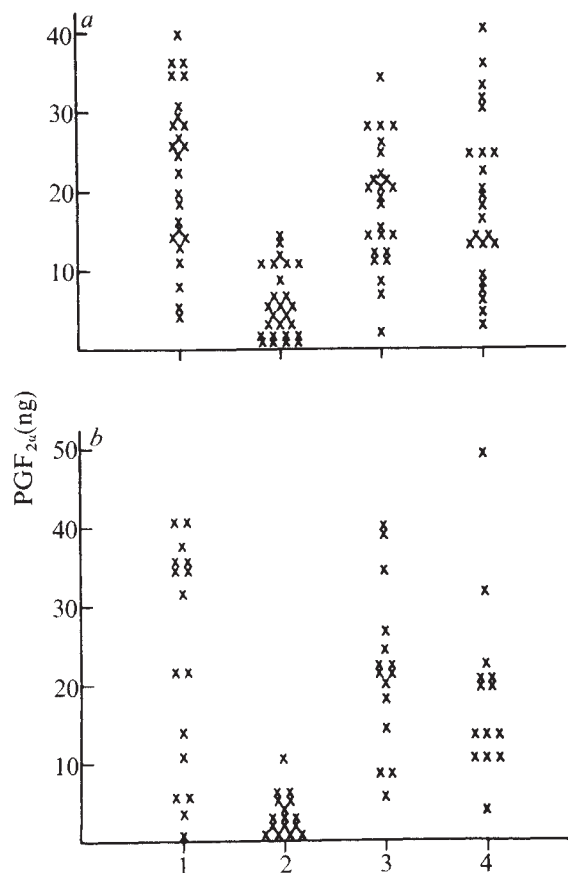


Fig. 2 Enzymatic production of PGF_{2α} by increments of cytoplasmic fractions from four muscle preparations from two chicks (*a*) and these same cytoplasmic fractions in the presence of a constant amount of PGE 9-ketoreductase purified from chicken heart (*b*). ×, Dystrophic breast muscle; Δ, normal breast muscle; ●, normal leg muscle; ○, symptom-free leg muscle from dystrophic chick; ●, normal breast muscle from adult chicken. Experimental conditions in *a* are the same as those described in Fig. 1. In *b*, increments of cytoplasmic fractions, purified PGE 9-ketoreductase, 1.0 μg PGE₂ and 1.0 mM NADPH in a volume of 0.1 ml, were incubated for 15 min at 37 °C. After boiling for 2 min followed by addition of 0.9 ml buffer and centrifugation, the supernatant fluid was assayed for PGF_{2α} by radioimmunoassay.

activity, presumably due to PGE 9-ketoreductase present in the extract, then actually decreased the reductase activity. In the presence of extracts of dystrophic muscles and the leg muscles, reductase activity was also increased; but with higher concentrations of these extracts, the degree of increase in reductase activity was reduced, and in the presence of 100 μl the reductase activity was even inhibited. If the inhibitor in the dystrophic breast muscle and the leg muscles is identical to that present in the normal breast muscle, the normal breast muscle contains 5 to possibly 50 times more of it. An inhibitor of PGE 9-ketoreductase has been partially purified from normal adult breast muscles of chickens. This inhibitor seems to contain a flavin-related moiety. Whether it is identical to the inhibitor found at reduced levels in dystrophic breast muscle is still to be demonstrated.

The effect of 50 μl of the four muscle extracts from several chicks on purified PGE 9-ketoreductase activity is shown in Fig. 3. Again it can be seen that levels of inhibition of the reductase by normal breast muscle are much higher than that of dystrophic breast muscles or the leg preparations. The findings

that the low activities of NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase in our experimental conditions are the same in normal and dystrophic breast muscle extracts suggest that the increased PGF_{2α} levels in the presence of the dystrophic muscle extracts did not reflect differences in enzymatic oxidation of the 15-hydroxy group.

The presence of different levels of inhibitor of PGE 9-ketoreductase can be added to the properties which distinguish the normal pectoralis (white) muscle from the thigh and leg (red) muscle of the chick⁷. In contrast, this distinction is absent in the dystrophic pectoralis which shows a low level of the inhibitor similar to that of the leg muscle. The properties which distinguish pectoralis (white) leg or heart (red) muscle appear during the last third of embryonic development and the first month of post-hatching. The retardation of the full development of the characteristics of white muscle in the dystrophic pectoralis has been demonstrated with regard to several cell properties⁸. Similarly, one could see in the absence of the PGE 9-ketoreductase inhibitor in dystrophic pectoralis another instance of a suppression of late development.

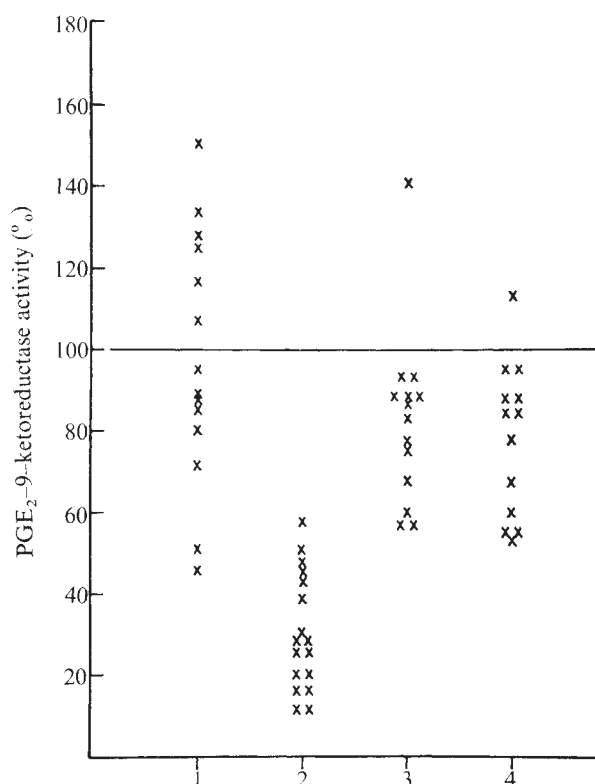


Fig. 3 PGE 9-ketoreductase activity of 50- μ l cytoplasmic fractions of the four muscle (for code, see Fig. 1) extracts from individual chicks in the presence of a constant amount of PGE 9-ketoreductase purified from chicken heart. Enzyme activity is expressed as percentage of that obtained with purified enzyme. Experimental conditions are the same as those in Fig. 2b.

Different profiles of prostaglandin metabolism have been found in the lung and kidney of foetal and early newborn rats and in adult rats^{9,10}. It is possible that the PG system, which includes generation of endoperoxides, thromboxanes, and the primary PGs, have an important role in differentiation. The fact that certain nutritional muscle dystrophies can be induced by a deficiency of an antioxidant such as tocopherol¹¹, that in these conditions peroxidation of lipids can be demonstrated¹², and that progress of chick dystrophy is retarded by sulphhydryl-protecting substances such as penicillamine¹³, could point to involvement of PG-derived peroxides in the dystrophic degeneration of chick breast muscle. How the relative levels of PGE and PGF_{2α} or the altered PGE 9-ketoreductase activity in the dystrophic breast muscle modulates the thromboxane pathway

or endoperoxide stability (if they do indeed have a regulatory role) is not clear.

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C fragment of lipotropin has a high affinity for brain opiate receptors

THE C-terminal region of lipotropin, named C fragment¹, has been found as an intact polypeptide in the pituitary gland² and the C fragment is also released from lipotropin by mild digestion with trypsin *in vitro*¹. These data suggest that the C fragment of lipotropin might be elaborated *in vivo* in order to perform a physiological function.

Recent reports have shown that in pituitary there is an endogenous peptide with opiate activity^{3,4} and similar peptides have been found in brain⁵⁻⁷. One of the brain peptides, termed methionine enkephalin, was isolated and described as a pentapeptide with the sequence Tyr-Gly-Gly-Phe-Met⁸, which is the same as the sequence at the NH₂-terminus of the C fragment of lipotropin. Although lipotropin⁹ and its C fragment^{1,2} were isolated from pituitary whereas the pentapeptide was isolated from brain⁶, it nevertheless seems probable that methionine enkephalin is derived *in vivo* from lipotropin C fragment. We have therefore determined whether C fragment, and a series of peptides that might be formed from C fragment *in vivo*, has a high affinity for brain opiate-binding sites. The results show that several of the peptides bind strongly to brain opiate receptors and, in particular, that C fragment has a higher affinity than the pentapeptide methionine enkephalin.

Lipotropin, C fragment (residues 61-91 of lipotropin), C' fragment (61-87), β -melanotropin (41-58) and N fragment (1-38) were isolated from pig pituitary as described previously¹. The peptide representing residues 61-89 was isolated from a carboxypeptidase A digest (Worthington) of C fragment. The octapeptide and nonapeptide representing residues 61-68 and 61-69 of lipotropin were obtained by enzymic digestion of C fragment with *Armillaria mellea*

protease (Arven) and TPCK-trypsin (Worthington) respectively; the peptides were isolated by ion-exchange chromatography on columns of Dowex 50-X2 with volatile pyridine buffers and were desalted on Sephadex G-10. The fragment containing residues 70-79 of lipotropin was isolated from the tryptic digest of C fragment. The peptides were characterised by N- and C-terminal analysis^{10,11} and by amino acid composition¹². The tetrapeptide and pentapeptide representing residues 61-64 and 61-65 of lipotropin were prepared by solid phase peptide synthesis¹³; the products were purified by chromatography on Sephadex C-25 and Sephadex A-25 and chemical and optical purity were confirmed by thin-layer chromatography and by digestion with aminopeptidase M (Röhm).

The peptides were tested for their ability to reduce the binding of the radiolabelled opiate agonist, ³H-dihydromorphine, and the antagonist ³H-naloxone to a membrane preparation from rat brain. The experimental details are given in Table 1. The concentrations of peptides that reduce the specific binding by 50% (*I*₅₀) are shown, together with the corresponding values for four opiate drugs. The determinations were made at 100 mM sodium concentration, conditions in which the *I*₅₀ values for displacement of bound naloxone have been shown to correlate with pharmacological potency¹⁴.

C fragment at low concentration was effective in displacing specifically bound ³H-naloxone. The *I*₅₀ value (2.6×10^{-9} M) was one tenth of that of morphine or dihydromorphine and was comparable with that of naloxone. Methionine enkephalin (61-65) and the intermediate peptides, 61-68, 61-69 and 61-87, were approximately 30 times less potent than C fragment. The *I*₅₀ values of the closely related peptides 61-87 and 61-89 differed by a factor of 20, indicating the importance of the paired lysine residues (88 and 89) to binding affinity. Lipotropin, which includes the C fragment sequence, and seems to be the precursor of C fragment¹⁵, did not inhibit the binding of ³H-naloxone or ³H-dihydromorphine, and fragments of lipotropin that lacked the C-terminal sequence were also inactive.

When the *I*₅₀ values were obtained from competition

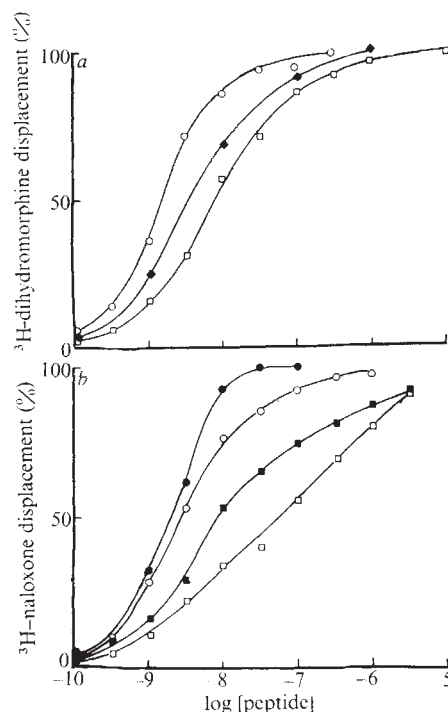


Fig. 1 *a*, Displacement of specifically bound ³H-dihydromorphine by C fragment (○), methionine enkephalin (□) and morphine (◆) in Tris-HCl, (pH 7.4), 100 mM NaCl. Incubation conditions are described in the legend to Table 1. *b*, Displacement of specifically bound ³H-naloxone by C fragment (circles) and methionine enkephalin (squares). Open symbols refer to incubations in Tris-HCl, (pH 7.4), 100 mM NaCl; closed symbols refer to incubations in Tris-HCl (pH 7.4), 100 mM KCl. KCl was included in the 'low sodium' incubation to distinguish the specific effects of sodium from nonspecific ionic strength effects. The effect of substitution of sodium by potassium in the incubation medium is to increase the specific ³H-dihydromorphine counts bound by 30-40% and to decrease the specific binding of ³H-naloxone by 50%. Note that, in general, these curves do not represent simple mass action binding processes.

Table 1 The binding of lipotropin fragments to brain opiate receptors

Residue Nos.	Name	against ³ H-naloxone M × 10 ⁹	<i>I</i> ₅₀ against ³ H-dihydromorphine M × 10 ⁹	Potency ratio: <i>I</i> ₅₀ naloxone
				<i>I</i> ₅₀ dihydromorphine
61-91	C fragment	2.6	2.2	1.2
61-89		5	2.2	2.3
61-87	C' fragment	100	42	2.5
61-69		60	21	2.9
61-68		160	45	3.6
61-65	Methionine enkephalin	90	8	11
61-64		1,000	300	3.3
1-91	β-LPH	≥ 1,000	≥ 1,000	—
1-58	γ-LPH	≥ 1,000	≥ 1,000	—
1-38	N-Fragment	≥ 1,000	≥ 1,000	—
41-58	β-MSH	≥ 1,000	≥ 1,000	—
70-79		≥ 1,000	≥ 1,000	—
	Morphine	25	4.1	6
	Levorphanol	10	2.0	5
	Dihydromorphine	22	3.6	6
	Naloxone	1.8	2.4	0.7

Opiate binding assays were carried out on a hypotonically lysed (10 mM Tris-HCl, pH 7.4), extensively washed, crude synaptosome preparation from whole supratentorial rat brain. The membranes (0.5 mg protein ml⁻¹) were preincubated for 15 min at 30 °C in a buffer containing 50 mM Tris-HCl (pH 7.4) and either 100 mM NaCl or KCl. Tritiated opiates (³H-naloxone (New England Nuclear, 20 Ci mmol⁻¹, 1.0×10^{-8} M) or 1,7,8-³H-dihydromorphine (Radiochemical Centre, Amersham, 90 Ci mmol⁻¹, 3.5×10^{-10} M) and appropriate concentrations of unlabelled opiates or peptides were added to 1-ml aliquots of preincubated membrane suspension in plastic microcentrifuge tubes. After 15 min incubation in the dark, the suspension was centrifuged (14,000g, 2 min), the pellets rapidly and superficially washed with 100 mM NaCl or KCl, and bound radioactivity assayed by liquid scintillation counting at 30% efficiency. Specific binding was defined as that fraction of the bound radioactivity displaced by morphine (10^{-6} M) or levorphanol (10^{-6} M). Incubations were carried out in quadruplicate and the standard error of the mean of a determination rarely exceeded 3%. Of 70,000 d.p.m. ³H-dihydromorphine in each assay, 2,700 d.p.m. are bound specifically and 1,500 d.p.m. bound nonspecifically. For ³H-naloxone, of the 46,000 d.p.m. in each incubation, 4,500 d.p.m. are bound specifically and 3,000 d.p.m. nonspecifically. The *I*₅₀ value represents the concentration of the compound at which 50% of specifically bound radioactivity is displaced.

experiments against ^3H -dihydromorphine (Table 1), C fragment was found to have twice the potency of morphine, and methionine enkephalin had approximately one half the potency (Fig. 1a). The tetrapeptide (61–64) and peptides intermediate between C fragment and methionine enkephalin were less potent than the pentapeptide. The active peptides and the opiate alkaloid agonists exhibited higher potencies when assayed against ^3H -dihydromorphine than against ^3H -naloxone. The change in I_{50} , reflected in the potency ratio (Table 1), was most marked in the case of methionine enkephalin and least for C fragment. Thus C fragment behaves more like the antagonist naloxone than the potent agonists morphine and levorphanol.

Replacement of sodium ions in the assay medium by potassium resulted in a large decrease (ninefold) in the I_{50} value for displacement of bound ^3H -naloxone by methionine enkephalin but had only a small effect (1.3-fold) on the I_{50} value of C fragment (Fig. 1b). With morphine-like alkaloids it has been observed that a large sodium effect correlates with agonist properties^{18–19}. Although such a correlation has not been demonstrated for opiate peptides, the results for methionine enkephalin agree with its reported agonist properties⁸. In contrast, C fragment exhibited a much smaller sodium effect. Experiments to test the pharmacological properties of C fragment are in progress²¹.

Preliminary studies of the time course of C fragment binding suggest that the peptide interacts with the receptor more slowly than dihydromorphine. Thereafter, the extent of inhibition of ^3H -dihydromorphine binding by C fragment remains approximately constant for 2 h. Inhibition by methionine enkephalin, on the other hand, decreases slowly after 20 min, consistent with slow proteolytic degradation of the pentapeptide. The possibility that C fragment might be weakly active but undergoes proteolytic cleavage to yield a more active peptide is unlikely because the 61–64, 61–65, 61–68, 61–69 and 61–87 peptides were less active than C fragment in the binding assay. Furthermore, degradation by a trypsin-like enzyme is highly unlikely because lipotropin, which readily undergoes cleavage *in vitro* to give C fragment¹, was inactive. Certainly, in our assay conditions, C fragment does not undergo conversion to methionine enkephalin as the binding properties of the two peptides are quite different.

The binding studies indicate that a series of peptides derived from lipotropin can inhibit the binding of naloxone and dihydromorphine to brain opiate receptors. The 'active' peptides all contain the N-terminal sequence Tyr–Gly–Gly–Phe, retaining a potential for the β -bend predicted for the receptor conformation of enkephalin²⁰. The most active peptide is C fragment, with an affinity substantially greater than that of methionine enkephalin. The high affinity exhibited by C fragment for brain opiate receptors *in vitro* suggests that this peptide, if present in higher regions of brain, could function *in vivo* as a neurotransmitter or neuro-modulator.

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Since this manuscript was submitted, the C fragment has been tested *in vivo* by Professor W. S. Feldberg using direct injection into the 3rd ventricle of the cat: it was found to have potent analgesic properties, the effects lasting several hours²¹. In molar terms, C fragment was approximately 200 times more potent than morphine.

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Structural and conformational relationships between the enkephalins and the opiates

Two pentapeptides, the enkephalins (Fig. 1a), have been isolated from the mammalian brain and have been shown to be potent opiate agonists^{1,2}. Further work has demonstrated that the opiate analgesics themselves interact competitively with the receptor sites for these endogenous pentapeptides. This explains in a simple and elegant manner the occurrence of opiate receptors in nervous tissue^{3,4}; clearly it was unlikely that they were there to interact solely with the opiates. As the enkephalins and opiates are competing for the same receptors and give a similar pharmacological response it is likely that they have structural and conformational similarities. Hughes *et al.* have shown that both of these small peptides contain a tyrosine residue at the amino terminal position⁵. We consider here the significance of that finding for the conformational relationship between the enkephalins and opiates.

The discovery that tyrosine is in a terminal position rather than in the internal sequence, where the nitrogen atom would be part of an amide linkage and therefore not basic, is of considerable significance. If we make the reasonable assumption that the primary locus of interaction of the above peptides with the opiate receptor is at the tyrosine terminal, then this would explain why the 'tyramine' portion of the tyrosine residue (Fig. 1a) is a common feature of many of the most potent groups of opiate analgesics such as the morphines (Fig. 1b), morphinans (Fig. 2a) and 6,7-benzomorphans (Fig. 2b). As several antagonists are derived from the above agonists similar considerations will also apply to these drugs. The 3-OH morphinans (Fig. 2a, levorphanol) and 2'-OH-6,7-benzomorphans (Fig. 2b, metazocine) are as potent as or more potent than morphine; therefore it is obvious that these simplifications of the morphine skeleton, which are considerable in the case of the benzomorphans, still contain the active moiety. The development of various elegant *in vitro*^{3,4} methods of assessing opiate agonist and antagonist activity has removed some of the problems of structure-activity correlations based simply on *in vivo* measurements where factors other than opiate receptor interaction may distort accurate interpretations. The fact that the tyramine fragment of the enkephalins has a primary amino group rather than a tertiary function as in many

opiates is perhaps not surprising. Normorphine, which contains a secondary amino group, is as active as morphine in various *in vivo*⁵ and *in vitro*⁶ systems, thus showing that the presence of a tertiary amino group is not an absolute requirement for agonist activity. The importance of the phenolic hydroxyl group in opiate agonists and antagonists is well known^{7,8}, and results from various *in vitro* systems³ show that the methyl ether of morphine, codeine, has only 0.7% of the potency of morphine in the guinea pig ileum and a mere 0.053% of its activity in the rat brain stereospecific binding assay, whereas as an analgesic in man it has about 8% of the activity of the parent drug. In the 6,7-benzomorphans series it has also been shown that replacement of the 2' phenolic-OH by $-\text{NO}_2$, $-\text{NH}_2$, Cl or F groups leads to less active agonists⁹.

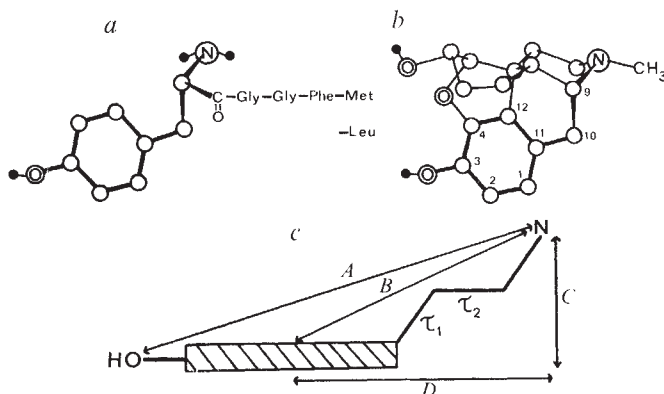


Fig. 1 *a*, Amino acid sequences of the enkephalin mixture² with the 'tyramine' moiety of the tyrosine residue in heavy outline. *b*, X-ray structure of $(-)$ -morphine·HI (ref. 17) with the tyramine moiety in heavy outline. *c*, Diagrammatic presentation of a side-on view of the tyramine moiety, the rectangle representing the benzene ring. *A*, Distance of the nitrogen atom from the oxygen atom; *B*, distance of the nitrogen atom from the centre of the aromatic ring; *C*, perpendicular distance of the nitrogen atom above the plane of the benzene ring; *D*, distance of the centre of the aromatic ring from the point of intersection of the plane of the benzene ring and the perpendicular distance *C*. The torsion angle τ_1 is defined as the angle between the planes of the atoms C11-C10-C9 and C11-C10-C9, that is, for rotation about the C11-C10 bond. Torsion angle τ_2 is the angle between the planes of the atoms C11-C10-C9 and C10-C9-N, that is, for rotation about the C10-C9 bond.

Although there is a 2-carbon chain between the benzene ring and the nitrogen atom there are also two possible 3-carbon chains between these two functions in the morphine, morphinan and 6,7-benzomorphans analogues; but preparation of simple 3-carbon amines carrying a *m*-phenolic-OH group produced drugs with only very weak analgesic activity¹⁰. As well as having a similar molecular fragment, most opiates exhibit stereospecificity of action⁷; essentially all the analgesic activity resides in the $(-)$ -isomer, and the configuration at C9 in $(-)$ -morphine and at the equivalent carbon atoms in the active morphinans and 6,7-benzomorphans is the same¹⁴.

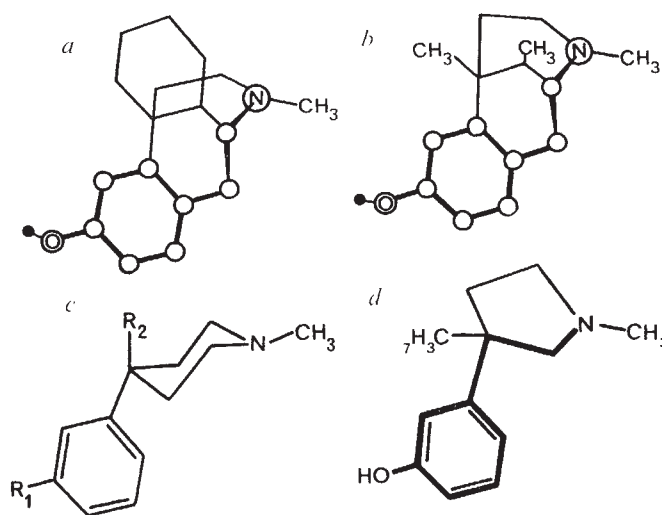


Fig. 2 The opiate agonists levorphanol (*a*) and metazocine (*b*) with the tyramine moiety in heavy outline; *c*, the 4-phenylpiperidines; *d*, profadol with the 2-carbon side chain amine and aromatic ring in heavy outline. O, carbon atoms; H, hydrogen atoms.

As the actions of the opiates are stereospecific and they are more or less rigid molecules, one possible way of obtaining information about the most likely opiate receptor conformation of the 'tyramine' portion of the tyrosine residue in the enkephalin pentapeptides is to examine the conformations of the tyramine residue in the potent opiates as determined by X-ray crystallography. The opiates are fairly rigid molecules so the conformation obtained from the X-ray data can confidently be assumed to be very similar to that occurring at the opiate receptor. We have therefore determined certain molecular parameters (Fig. 1c and Table 1) for the tyramine fragment in several conformationally restricted opiate agonists and antagonists based on published X-ray data. It is probably reasonable to examine both opiate agonists and antagonists, as there is now excellent evidence that they are combining with the same or very similar receptor sites^{3,4,6,11,12}. For the agonists morphine and codeine, the antagonists, naloxone and 2-allyl 5,9-dimethyl-2'-hydroxy 6,7-benzomorphans¹³ (*N*-allylnormetazocine) and the agonist-antagonist⁶ cyclazocine (*N*-cyclopropylmethylnormetazocine) we find the following mean values for the various molecular parameters $A = 7.0 \text{ \AA}$, $B = 4.5 \text{ \AA}$, $C = 1.2 \text{ \AA}$, $D = 4.4 \text{ \AA}$, $\tau_1 = 175^\circ$, $\tau_2 = -89^\circ$. A combination of some of these or closely similar values may correspond to the optimal opiate receptor site conformation for the 'tyramine' fragment of the enkephalins. Further simplifications of the morphine structure has led to molecules having greater degrees of conformational freedom, such as the 4-phenylpiperidines (Fig. 2c) and the 3-phenylpyrrolidines (Fig. 2d). The former group of drugs is

Table 1 Molecular parameters for various opiate agonists, antagonists and other drugs

	<i>A</i> (Å)	<i>B</i> (Å)	<i>C</i> (Å)	<i>D</i> (Å)	τ_1 (degrees)	τ_2 (degrees)
$(-)$ -Morphine·HI ¹⁷	7.08	4.55	0.81	4.47	164.7	-94.9
$(-)$ -Codeine·HBr ¹⁸	7.06	4.54	0.88	4.45	171.9	-91.4
$(-)$ -Naloxone·HCl ¹⁹	6.96	4.32	1.27	4.12	180.7	-87.3
$(\pm)$ -2-Allyl 5,9-dimethyl-2'-hydroxy 6,7-benzomorphans ·HBr ²⁰ (<i>N</i> -allylnormetazocine)	6.89	4.42	1.74	4.06	174.2	-81.3
$(-)$ -Cyclazocine·HBr ²¹ (<i>N</i> -cyclopropylmethyl normetazocine)	7.08	4.55	1.23	4.38	182.6	-94.2
Meperidine HBr ²²		5.71	1.24	5.57		
Tyramine HCl ²³	7.82	5.17	1.36	4.99	109.3	181.3
L-Tyrosine ethyl ester ²⁴	7.91	5.19	1.32	5.02	108.1	179.9

The molecular parameters are defined in the legend to Fig. 1. Values *A*, *B*, *C*, τ_1 and τ_2 were all calculated from the published atomic coordinates; the values for *D* were obtained by simple geometry.

based on the piperidine-phenol ring fragment in morphine and it is, therefore, not surprising that the optimal position for the hydroxyl group is *meta* with respect to the piperidine ring¹⁴. The most active drugs of the 3-phenylpyrrolidine series also have the hydroxyl group in the *meta* position¹⁵. Although in the tyramine fragment of morphine the hydroxyl group is *para*, models of the 3-phenylpyrrolidines clearly show that the spatial disposition of the nitrogen atom with respect to the benzene ring and hydroxyl group is very similar to that in morphine.

Similar considerations apply to the *m* hydroxyl 4-phenylpiperidines although here models and the *B* distance in mepe-

ridine (Fig. 2c, $R_1 = H$, $R_2 = -\overset{\text{O}}{\parallel}\text{C}-\text{OC}_2\text{H}_5$) indicate that the distances differ from those found for the more rigid opiates. Two of the more widely investigated drugs in this group are the

agonists bemidone (Fig. 2c, $R_1 = OH$, $R_2 = -\overset{\text{O}}{\parallel}\text{C}-\text{OC}_2\text{H}_5$) and (–)-profadol (Fig. 2d). In *in vitro*^{3,6} systems the former has $1/12$ and the latter $1/9$ of the potency of (–)-morphine. It is therefore tempting to ascribe this decrease of potency to the greater degree of conformational mobility and to the fact that the various moieties may not be held in their optimal positions as in the more rigid opiates. This simple concept is complicated, however, by the fact that ketobemidone (Fig. 2c,

$R_1 = OH$, $R_2 = -\overset{\text{O}}{\parallel}\text{C}-\text{C}_2\text{H}_5$) both in *in vivo*^{7,14} and *in vitro*³ tests has a similar potency to morphine; the conformation of this drug, in the crystal state and in solution, is worthy of further study. Clearly however, with these less rigid drugs it is unwise to place too much importance on the possible pharmacological significance of values obtained by these methods.

It must be stressed, however, that although the presence of the tyramine fragment may be of importance in partially explaining the action of several groups of agonists and antagonists the stereospecificity/selectivity factor and the contribution of additional modes of binding from other portions of the molecule are obviously also critical⁸. The fact that drugs such as methadone and dextromoramide are quite different from those discussed here, and do not contain a tyramine fragment yet have agonist activities equal to or greater than morphine⁷ and are probably acting on the same receptor^{3,6,16}, reinforces the above suggestions that other structural factors and modes of receptor interaction are obviously also important.

It is well known⁷ that the introduction of certain substituents on to the N atom converts the opiates into antagonists, for example, substitution of an allyl group for the methyl group on nitrogen converts the agonists morphine and oxymorphone into the antagonists nalorphine and naloxone, respectively. It would be of interest to see what effect similar substitutions on the nitrogen of the tyramine fragment of the enkephalins produced, that is, would this result in antagonists or just less active molecules? Until there are data on the crystal and solution conformations of the enkephalins and the results of varying the structure are known, it would be premature to speculate on the significance of the rest of the peptide sequence. It seems worthy of emphasis, however, that the 'tyramine' conformation we have suggested might be the preferred one, for these pentapeptides at opiate receptors may not correspond to the preferred conformation for this residue found by X-ray crystallography, NMR spectroscopy or theoretical calculations, because the enkephalin pentapeptides are flexible molecules unlike the opiates and close analogues which are fairly rigid. In this regard, it is of interest that both tyramine HCl and tyrosine ethyl ester in the crystalline state have similar values for some of the molecular parameters (Table 1) but that these differ from those found for the opiates.

After this paper had been accepted for publication an article by Bradbury *et al.* appeared²⁵ in which they suggested that

methionine enkephalin bears no similarity in its primary structure to the opiates; we feel, however, that our theory argues strongly against this.

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Increased membrane fluidity implicated in acceleration of decay of post-tetanic potentiation by alcohols

AFTER repetitive stimulation of a synapse, the efficacy of synaptic transmission may be potentiated for a period. In systems that lend themselves to detailed analyses, this post-tetanic potentiation (PTP) has been shown to be due to an increased amount of transmitter released by the presynaptic neurone rather than to postsynaptic mechanisms¹. We have investigated the PTP of a monosynaptic, all-or-none, excitatory postsynaptic potential (e.p.s.p.) recorded in cell R15 of the abdominal ganglion of *Aplysia californica* with appropriate stimulation of the right visceropleural connective, and have presented evidence that the PTP of this e.p.s.p. is based on increased transmitter release². We have shown³ that while PTP is slightly prolonged by lowering the temperature of the preparation from 20 to 12 °C, the duration of the PTP is increased tenfold by further lowering of the temperature to 10 °C. This demonstration of a transition temperature suggested that the fluidity of critical lipids in the presynaptic membrane regulates the decay of PTP, such that PTP decays more slowly in conditions of decreased membrane fluidity. If this inference is correct, agents, such as alcohols⁴, that increase membrane fluidity and thus act as 'antifreezes', should either prevent the occurrence of the temperature transition or lower the transition temperature. These agents should also, by themselves, accelerate the rate of PTP decay and their potency should be correlated with their lipophilicity. We present here experimental evidence in support of these predictions.

The abdominal ganglion of *A. californica* was pinned in a dish and perfused with fortified artificial seawater at 15 °C

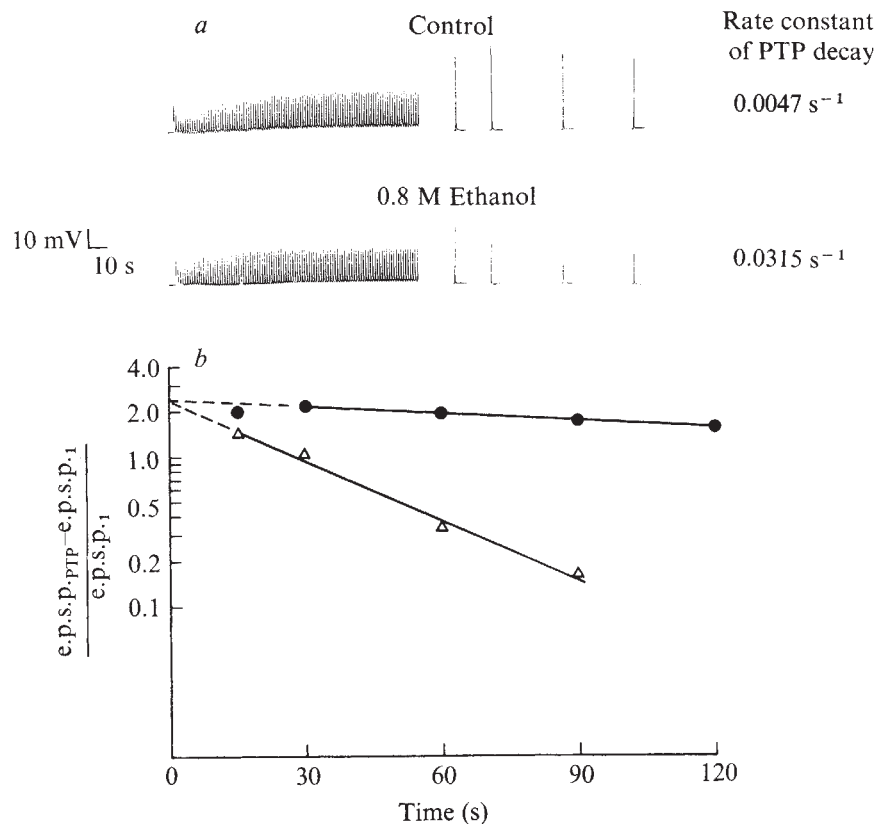


Fig. 1 Bath application of 0.8 M ethanol increases the rate constant of the decay of PTP. *a*, Intracellular record of the e.p.s.p.s recorded in cell R15 of the abdominal ganglion on stimulation of the right visceropleural connective during a train of 100 pulses at 1 per s followed by test pulses at 15-s intervals and then every 30 s. Cell R15 was hyperpolarised to -100 mV by injecting current through the second barrel of a double-barrelled recording electrode. Repetitive stimulation at 1 pulse per s resulted in an initial decrease in the size of the e.p.s.p. (synaptic depression) followed by a gradual increase of the e.p.s.p. amplitude to a facilitated plateau (frequency facilitation). When the train stopped, PTP of the e.p.s.p. amplitude was observed. Thirty minutes after a control train ethanol perfusion was begun and a train was given in the presence of ethanol 30 min later. Ethanol had no effect on the amplitude of the e.p.s.p.s during the train but selectively shortened the duration of PTP. *b*, During PTP, the amplitude of the test e.p.s.p. (e.p.s.p._{PTP}) returned towards the size of the first e.p.s.p. of a train (e.p.s.p.₁) with a single exponential time course. The rate constant of the decay of PTP (the slope of the straight line fitted to the data points after the peak by linear regression analyses) was increased by the application of ethanol. The zero time intercept (a measure of the PTP amplitude) was not affected significantly by ethanol. Data are from a single preparation. ●, Control; △, 0.8 M ethanol.

(ref. 5) to which alcohols were added when indicated. Cell R15 was hyperpolarised to eliminate endogenous bursting; stimulation and intracellular recording were as before^{2,3}. The right visceropleural connective was stimulated with trains of 100 pulses at 1 s^{-1} . On termination of the train, two test pulses were given at 15-s intervals to obtain an approximation of the maximal amplitude of the e.p.s.p. during the PTP period. Thereafter test pulses were given at 30-s intervals to obtain an estimate of the duration of PTP.

Perfusion of the preparation with 0.8 or 1.0 M ethanol (in fortified artificial seawater³) greatly accelerated the decay of PTP (Fig. 1 and Table 1). The effect on PTP decay was selective, since, on the average, ethanol had no effect on an isolated e.p.s.p., on synaptic depression (the ratio of the amplitudes of the first to the second of a pair of e.p.s.p.s 1 s apart), on frequency facilitation (the ratio of the last to the first e.p.s.p. of a train) or on the maximum amplitude of the PTP (the zero time intercept of plots like that of Fig. 1*b*) (Table 1). Ethanol has also been seen to increase the rate of decay of PTP at a mammalian neuromuscular junction¹.

We tested the effect of ethanol on the transition temperature below which PTP decays very slowly³. In three different experiments, lowering the temperature from 20°C to 8°C , in the presence of 0.8 M ethanol, only slightly increased the duration of the PTP, and no evidence was found for the existence of a transition temperature between 12°C and

10°C . In each of these three preparations the ethanol was subsequently removed and the duration of the PTP was determined at 12°C and 10°C . In the absence of ethanol the PTP was strikingly prolonged on lowering the temperature from 12°C to 10°C as had been found before³. The fact that ethanol, an agent which promotes membrane fluidity⁴, prevents the effect of lowering temperature, supports our conclusion³ that the transition temperature reflects the sudden decrease in the fluidity of critical presynaptic membrane components.

The inference that ethanol accelerates the rate of decay of PTP by promoting membrane fluidity was further supported by the progressive increase in potency of the series of aliphatic alcohols (ethanol to *n*-octanol) in accelerating the decay of PTP (Fig. 2*a*). The relative potency of the alcohols correlated with their partition coefficient in an *n*-octanol-water system⁶ (Fig. 2*b*).

These findings, that alcohols accelerate the decay of PTP, and prevent the previously observed³ temperature transition in the rate of PTP decay, suggest that the rate is limited by the membrane fluidity of some nerve terminal component. How direct an effect membrane fluidity has on PTP decay rate is unknown. The effect could be fairly indirect. For example, increasing membrane fluidity may activate a membrane-bound enzyme which in turn promotes the decay of PTP. Alternatively, the change in efficiency of transmitter release which directly underlines PTP^{2,7,8} may itself be a

Table 1 Effect of ethanol on an isolated e.p.s.p., synaptic depression, frequency facilitation and PTP

	Isolated e.p.s.p. (mV)	Synaptic depression	Frequency facilitation	PTP	Rate constant of decay of PTP (s^{-1})
Control $\pm$ s.e.	12.6 ± 2.4	1.11 ± 0.03	1.71 ± 0.23	0.72 ± 0.22	0.0038 ± 0.0006
1.0 M ethanol $\pm$ s.e.	13.2 ± 1.9	1.10 ± 0.03	1.40 ± 0.09	0.76 ± 0.26	0.0245 ± 0.0064
<i>P</i> (paired <i>t</i> test, $n = 11$)	NS	NS	NS	NS	< 0.005

Synaptic depression is defined as the ratio of the amplitudes of the first to the second e.p.s.p.s of a train elicited at a frequency of 1 s^{-1} . Frequency facilitation is the ratio of the amplitudes of the last to the first e.p.s.p. of a train of 100 pulses at 1 s^{-1} . To determine the amplitude of PTP and the rate constant of decay of PTP, the first four test e.p.s.p.s given at 30-s intervals starting 15 s after the end of a train were plotted as in Fig. 1*b*. A straight line was fitted to these points by linear regression analyses. The zero time intercept is a measure of the PTP amplitude and the slope of the line represents the rate constant of decay of PTP. NS, not significant.

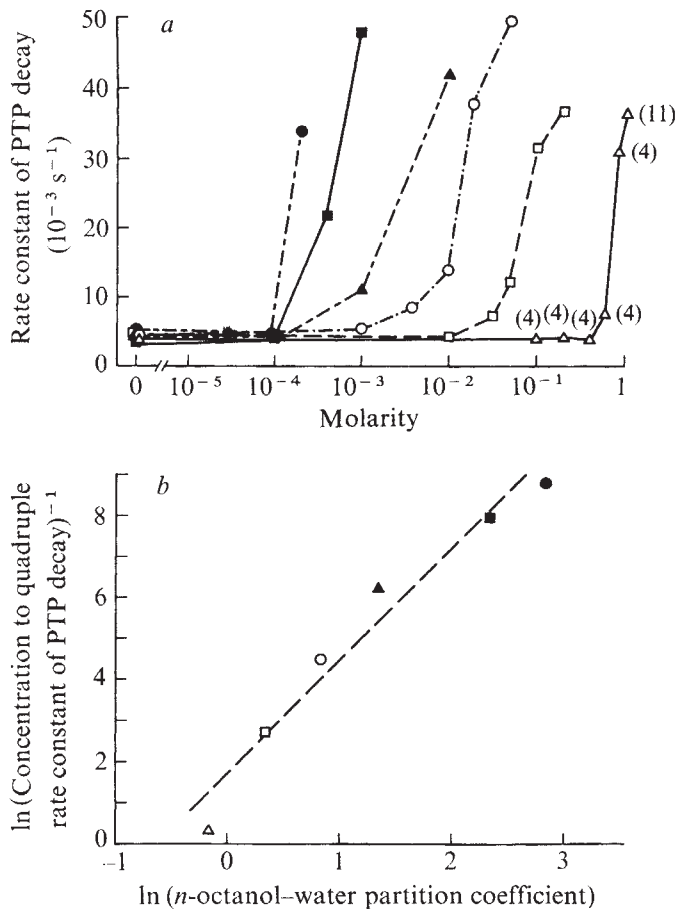


Fig. 2 *a*, Potency of aliphatic alcohols in increasing the rate constant of PTP decay. $\bullet$, C_8 ; $\blacksquare$, C_7 ; $\blacktriangle$, C_5 ; $\circ$, C_4 ; $\square$, C_3 ; $\triangle$, C_2 . The size of the carbon chain is indicated by the number (C_2 is ethanol; C_8 is *n*-octanol). An aliphatic alcohol (in fortified artificial seawater) was perfused for 30 min, beginning 30 min after a control train; and then a train was given in the presence of the alcohol. In contrast with ethanol, the higher alcohols reduced the amplitudes of all e.p.s.p.s. PTP still decayed, however, with a single exponential time course with a rate constant which was readily determined. Each data point represents the average value obtained from three different preparations, except for ethanol where the number of preparations at each data point is indicated. Note that the effect of each alcohol increases markedly over a narrow range of concentration. *b*, The potency of the effect of the different alcohols (measured as the concentration, C , needed to increase the rate of decay of PTP fourfold) correlates with the lipophilicity of the alcohol (as measured by the *n*-octanol: water partition coefficient, P). The data points were obtained from (a) by interpolating the concentration of each alcohol which would give a rate constant of PTP decay of $20 \times 10^{-3} \text{ s}^{-1}$. A plot of $\ln(1/C)$ against $\ln P$ gives a straight line with the equation $\ln(1/C) = 2.71 \ln P + 1.66$. Other biological systems susceptible to alcohols similarly give straight lines in these coordinates⁶. Symbols as in (a).

change in presynaptic membrane organisation (for example, a change in the number or effectiveness of "vesicle attachment sites"). In this latter case, the relaxation of this state of organisation would correspond to the decay of PTP, and would be directly affected by the fluidity of components of the presynaptic membrane. Evidence that this possibility is consistent with other known properties of PTP will be presented later. Regardless of the steps connecting membrane fluidity to the rate of PTP decay, our findings suggest that nerve terminal membrane fluidity is a potential physiological regulation point for the duration of PTP.

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Single-channel currents recorded from membrane of denervated frog muscle fibres

THE ionic channel associated with the acetylcholine (ACh) receptor at the neuromuscular junction of skeletal muscle fibres is probably the best described channel in biological membranes. Nevertheless, the properties of individual channels are still unknown, as previous studies were concerned with average population properties. Macroscopic conductance fluctuations occurring in the presence of ACh were analysed to provide estimates for single channel conductance and mean open times¹⁻³. The values obtained, however, depended on assumptions about the shape of the elementary conductance contribution—for example, that the elementary contribution is a square pulse-like event². Clearly, it would be of great interest to refine techniques of conductance measurement in order to resolve discrete changes in conductance which are expected to occur when single channels open or close. This has not been possible so far because of excessive extraneous background noise. We report on a more sensitive method of conductance measurement, which, in appropriate conditions, reveals discrete changes in conductance that show many of the features that have been postulated for single ionic channels.

The key to the high resolution in the present experiments lies in limiting the membrane area from which current is measured to a small patch, and thereby decreasing background membrane noise. This is achieved by applying closely the tip of a glass pipette, 3-5 μm in diameter, on to the muscle surface, thus isolating electrically a small patch of membrane (Fig. 1). This method has been applied previously in various modifications and mostly with larger pipette tips to muscle⁴, molluscan neurones^{5,6}, and squid axon⁷. The pipette, which has fire-polished edges, is filled with Ringer's solution and contains the cholinergic agonist at micromolar concentrations. Its interior is connected to the input of a virtual-ground circuit, which clamps the potential inside the pipette to ground and at the same time measures current flowing through the pipette, that is, through the patch of membrane covered by the pipette opening. The interior of the muscle fibre is clamped locally to a fixed value by a conventional two-microelectrode clamp⁸. Thus, voltage-clamp conditions are secured across the patch of membrane under investigation. Since current

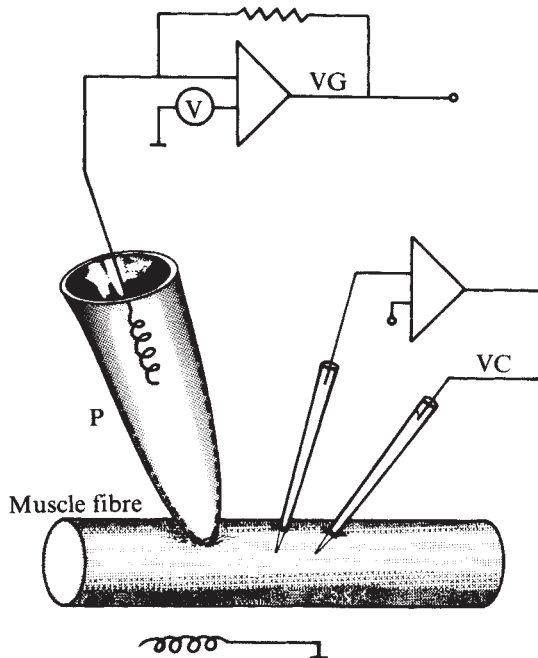


Fig. 1 Schematic circuit diagram for current recording from a patch of membrane with an extracellular pipette. VC, Standard two-microelectrode voltage clamp circuit to set locally the membrane potential of the fibre to a fixed value. P, Pipette, fire polished, with 3–5 μm diameter opening, containing Ringer's solution and agonist at concentrations between 2×10^{-7} and 6×10^{-6} M. d.c. resistance of the pipette: 2–5 M Ω . The pipette tip applied closely on to the muscle fibre within 200 μm of the intracellular clamp electrodes. VG, Virtual ground circuit, using a Function Modules Model 380K operational amplifier and a 500 M Ω feedback resistor to measure membrane current. The amplifier is mounted together with a shielded pipette holder on a motor-driven micromanipulator. V, Bucking potential and test signal for balancing of pipette leakage and measuring pipette resistance.

densities involved are very small, a simple virtual ground inside the pipette is preferable to more complicated arrangements for stabilising potential described previously⁸.

The dominant source of background noise in these measurements was the leakage shunt under the pipette rim between membrane and glass. It was constantly monitored by measuring the electrical conductance between pipette interior and bath. Discrete conductance changes could be resolved only when the conductance between pipette interior and bath decreased by a factor of four or more after contact between pipette and membrane. To minimise the leakage conductance, the muscle was treated with collagenase and protease⁹. This enzyme treatment digested connective tissue and the basement membrane, thereby enabling closer contact between glass and membrane. At the same time, however, it made the membrane fragile and more sensitive to damage by the approaching pipette. It

did not, however, change the ACh sensitivity of the fibre or alter the properties of ACh-induced conductance fluctuations (E.N. and B.S., unpublished).

All experiments were carried out on the extrasynaptic region of denervated hypersensitive muscle fibres. The uniform ACh sensitivity found over most of the surface of these fibres greatly enhanced the probability of the occurrence of agonist-induced conductance changes at the membrane patch under investigation. Extrasynaptic ACh channels of denervated muscle fibres have mean open times which are about three to five times longer than those of endplate channels^{1,10–12}. The longer duration facilitated the detection of conductance changes. Additional measures were taken which are known to either increase the size of the elementary current pulse or prolong its duration: the membrane was hyperpolarised up to –120 mV; suberyldicholine (SubCh) was used as an agonist in most of the experiments; the preparation was cooled to 6–8 °C.

Figure 2 shows a current recording taken in the conditions outlined above. Current can be seen to switch repeatedly between different levels. The discrete changes are interpreted as the result of opening and closing of individual channels. This interpretation is based on the very close similarity to single-channel recordings obtained in artificial membrane systems¹³. The preparation under study is, however, subject to a number of additional sources of artefact. Therefore it is necessary to prove that the recorded events do show the properties which are assigned to ionic channels of the cholinergic system. These are: a correlation with the degree of hypersensitivity of the muscle membrane; an amplitude dependent on membrane potential as predicted by noise analysis; a mean length or channel open time, which should depend on voltage in a characteristic manner²; pharmacological specificity with different mean open times for different cholinergic agonists^{14,15}. The experiments bore out all of the above-mentioned points as outlined below.

The frequency of occurrence of single blips depended on the sensitivity of the patch under investigation. A plot of the number of current pulses per second against the iontophoretically measured sensitivity of the membrane region, determined either immediately before or after the pipette experiment, revealed a distinct correlation between both quantities with a correlation coefficient of 0.91 for a linear regression (Fig. 3b). Student's *t* test assigned a significance better than 0.1% to the relationship.

To estimate the size of the current pulses amplitude histograms were calculated from the current recordings (Fig. 3c). The histograms show a prominent peak of gaussian shape around zero deviation, the width of which is a measure of the high frequency background noise of the current trace. Multiple, equally spaced peaks at larger deviations represent the probabilities that either one, two, or three channels are open simultaneously. The peak separation gives the amplitude of the single-channel contribution, which was 3.4 pA for the histogram shown in Fig. 3c.

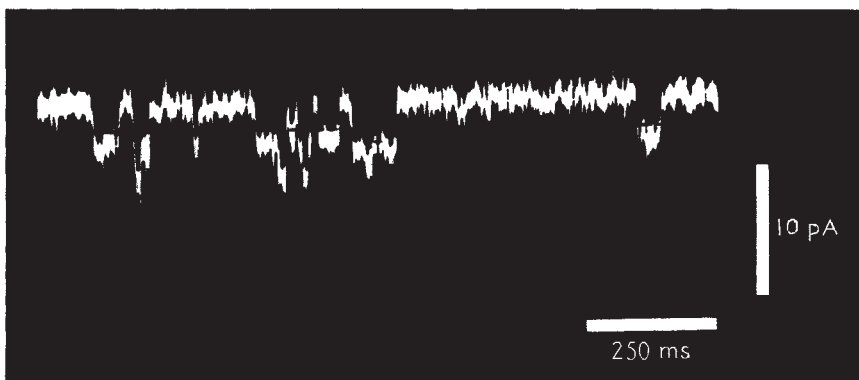
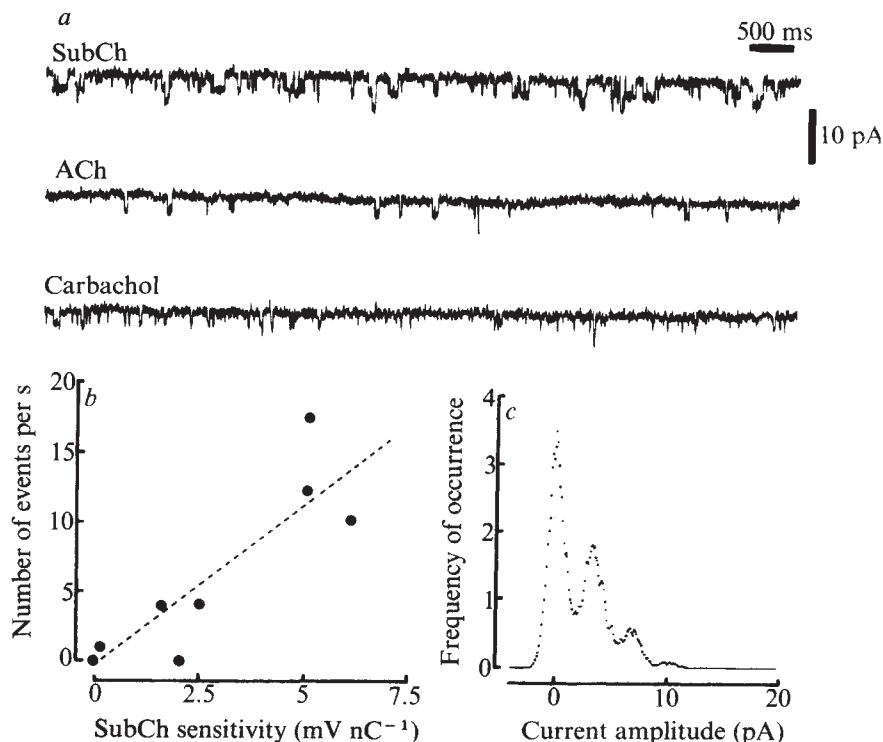


Fig. 2 Oscilloscope recording of current through a patch of membrane of approximately 10 μm^2 . Downward deflection of the trace represents inward current. The pipette contained 2×10^{-7} M SubCh in Ringer's solution. The experiment was carried out with a denervated hypersensitive frog cutaneous pectoris (*Rana pipiens*) muscle in normal frog Ringer's solution. The record was filtered at a bandwidth of 200 Hz. Membrane potential: –120 mV. Temperature: 8 °C.

Fig. 3 Characterisation of single-channel currents. *a*, Comparison of current recordings obtained with different cholinergic agonists at concentrations of 2.10^{-7} M (SubCh), 2.10^{-6} M (ACh), and 6.10^{-6} M (carbachol). Downward deflection of the trace represents inward current. Three different experiments. Pen records replayed from analogue tape at a bandwidth of 100 Hz. All experiments at -120 mV membrane potential; 8°C . *b*, Number of current blips per second is plotted against iontophoretic sensitivity of the membrane region under investigation. Sensitivity was determined by 100-ms iontophoretic pulses delivered from a pipette filled with 1 M SubCh ($40\text{ M}\Omega$ pipette resistance, 10 nA bucking current, sensitivity measured at resting potential). Pooled data from eight experiments. Broken line represents linear regression. *c*, Amplitude histogram of membrane current. Current traces were digitalised and baselines fitted by eye to data records, each 4 s in length. Frequency of occurrence of deviations from the baseline is shown in arbitrary units. Histograms were calculated on a PDP-11 computer; 2–8 histograms were averaged to obtain curves like the one shown. 8°C ; -120 mV membrane potential.



This was obtained from an experiment at -120 mV membrane potential. A similar histogram from the same muscle fibre obtained at -80 mV yields a current pulse amplitude of 2.2 pA. These two values extrapolate to an equilibrium potential of -7 mV. Channel conductance is estimated as 28 pmhos in this case. It scattered from fibre to fibre with a mean value of 22.4 ± 0.3 pmhos (mean $\pm$ s.e.; number of determinations = 27). This value is somewhat lower than the one derived from noise analysis at normal endplates, which is 28.6 pmhos for SubCh¹⁵. Higher order peaks in the histograms are not merely scaled images of the zero order peak. They tend to be smeared out due to non-uniformity of current pulse amplitudes. We cannot decide at present whether this is a real feature of the channels or a measurement artefact. Such an effect could arise if not all of the channels are located ideally in the central region of the pipette opening. Current contributions from peripherally located channels would only partially be picked up by the pipette. This source of error is also likely to lead to an underestimate of channel size if the pipette seal is not optimal.

Temporal analysis of the current records was carried out partly by measurement of individual channel length and averaging 40–50 measurements, and partly by calculation of the power spectrum of the current recordings. In the latter case, the cutoff frequency f_0 of the Lorentzian spectrum yielded an estimate of mean channel open time τ (or pulse duration) through the relationship $\tau = 1/(2\pi f_0)$. Values of mean open times obtained by the two methods were consistent within $\pm 30\%$. For SubCh as an agonist and at a temperature of 8°C it was 45 ± 3 ms ($n=11$) at -120 mV and 28 ± 3 ms ($n=14$) at -80 mV. These values are approximately three times longer than the corresponding mean open times of endplate channels derived from noise analysis¹⁵. Note, however, that lengthening of channel durations by factors of three to five at extrajunctional sites with respect to endplate values has been measured independently by conventional noise analysis¹². The voltage dependence of the values given above corresponds to an e -fold change per 80 mV, which is within the range of published values¹.

Channel open times were different when different cholinergic agonists were used (Fig. 3*a*). For -120 mV and 8°C , mean channel open time was 45 ± 3 ms ($n=11$) for SubCh, 26 ± 5 ms ($n=4$) for ACh, and 11 ± 2 ms ($n=3$) for carbachol. This sequence reflects the well known relationship between the open times of channels induced by these drugs at normal endplates^{14,15} and at extrasynaptic membrane of hypersensitive fibres¹².

The results obtained so far, especially the pharmacological specificity, lead us to conclude that the observed conductance changes are indeed recordings of single-channel currents. They are consistent with the conclusions drawn from statistical analysis of endplate current fluctuations, and show that current contributions of individual channels are of the form of square pulses. In addition, analysis of areas under the peaks of histograms like Fig. 3*c* indicates that in our experimental conditions opening of individual channels is statistically independent, since the probabilities of zero, one, or two channels being open simultaneously follow—within the limits of experimental resolution—a Poisson distribution.

Recordings of single-channel currents finally resolves the third level of quantification in the process of neuromuscular transmission after the discovery of endplate currents and miniature endplate currents. It should facilitate discrimination between factors influencing the properties of single channels and agents creating or modifying different populations of channels.

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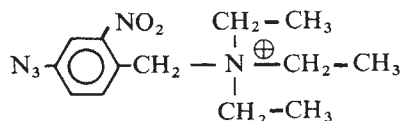
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Selective inhibition of potassium conductance in node of Ranvier with a photoaffinity label derived from tetraethylammonium

BIOCHEMICAL investigation of membrane components involved in ion-specific conductance changes is difficult because of the lack of a functional assay for the components after membrane solubilisation. Proteins thought to participate in conductivity changes during synaptic transmission¹ have been isolated using a binding assay and affinity chromatography^{2,3}, because of their high affinity for a variety of ligands; but the connection between such receptor proteins and postulated ion channels remains to be shown. To isolate molecules forming sodium channels of the axon membrane^{4–7}, tetrodotoxin was used as ligand (binding assay $K_d = 3$ nM). The most efficient known blocking agent of the change in potassium conductance in nerve and muscle known is tetraethylammonium (TEA) but its affinity is too low ($K_d = 0.4$ mM (ref. 8)) for either of the above techniques. We have attempted to label radioactively those molecules associated with the voltage-dependent potassium conductance changes covalently *in situ* with the aim of recovering that radioactivity after solubilisation using classical fractionation procedures. We suggest that it is possible to block selectively and irreversibly potassium conductance in myelinated nerve fibres using a photoaffinity label. This method may open the way for the isolation of the TEA receptor of excitable membranes, proposed as part of the potassium channel (if not the channel itself).

We applied 4-azido-2-nitrobenzyltriethylammonium fluoroborate,



which belongs to a class of photoaffinity labels developed by H.K.⁹ and which was first used in the investigation of the structure of the acetylcholine receptor (F.H., P. Layer, H.K. and G. Bandini, unpublished) and the acetylcholine esterase (P. Layer, H.K. and F.H., unpublished). The label was synthesised using the same procedure described for the corresponding trimethylammonium derivative (P. Layer, H.K. and F.H., unpublished). Voltage clamp experiments were carried out with sensory and motor fibres of the sciatic nerve of the frog *Rana esculenta*^{10,11}. The Ranvier node was continuously superfused with Ringer or the test solution as indicated in Figs 1–4. Photoaffinity labelling was induced by irradiation with an ultraviolet lamp Typ TL-900 (Camag, Switzerland; emission maximum 254 nm; lamp distance from fibre 5 cm). Without irradiation the photoaffinity label reversibly inhibited the potassium conductance (Fig. 1c), whereas the sodium conductance was not affected.

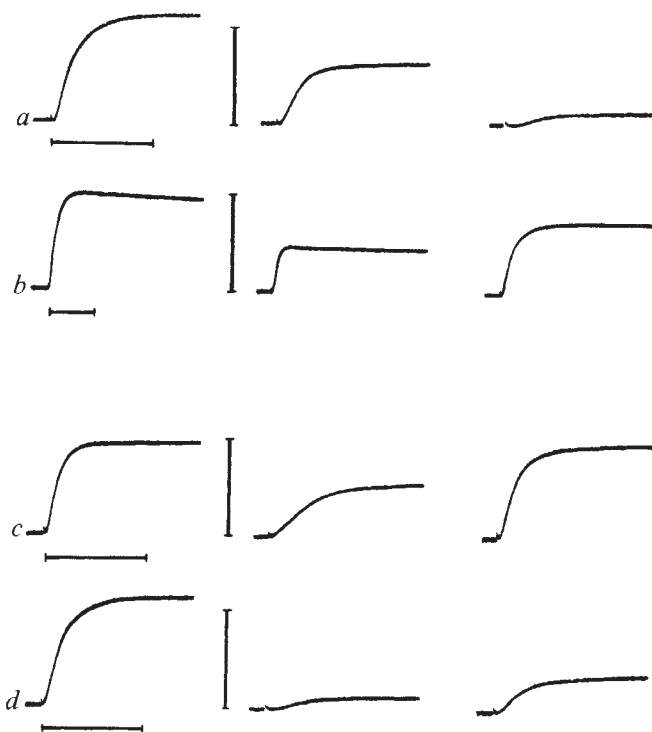
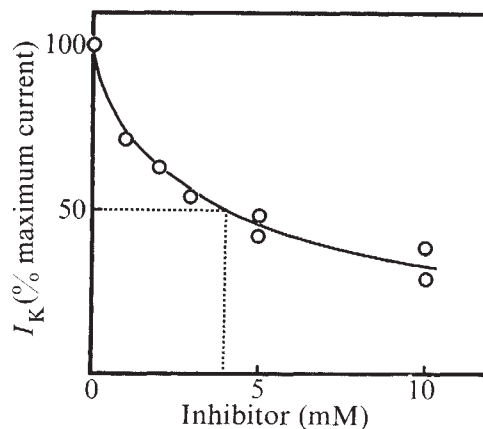


Fig. 1 Potassium current records during 140 mV depolarising pulses from a holding potential of -100 mV. Capacity and leakage currents were compensated analogically. Test node superfused with Ringer's solution (117 mM NaCl, 2.5 mM KCl, 1.8 mM CaCl_2) plus tetrodotoxin (300 nM). Control, First record in each row. In each experiment photoaffinity label (1 mM) was added either to Ringer's solution (*a*, *c* and *d*) or to isosmotic KCl solution in contact with the cut ends of the fibre (*b*). *a* and *b*, Effects of photoaffinity label applied either externally (*a*) or internally (*b*), before (second records) or after (third records) application of ultraviolet light for 3 min. *c* and *d*, Reversibility for the experiment without (*c*) or with irradiation (*d*). Third record in *c* and *d* was taken 40 min after removal of photoaffinity label from Ringer's solution. Vertical and horizontal calibrations: 10 nA and 10 ms, respectively.

50% inhibition of the potassium current was obtained with 4 mM photoaffinity label (Fig. 2), indicating a much lower affinity than that for TEA ($K_d = 0.4$ mM (ref. 8)). During irradiation in the presence of the label the potassium current decreased markedly and rapidly (Figs 1a and 3), whereas the sodium current decreased only slightly (Fig. 3). In a typical experiment, we obtained after about 2 min irradiation an 80% decrease in potassium current and a less than 10% decrease in sodium current. After removal of the

Fig. 2 Dose-response curve for 4-azido-2-nitrobenzyltriethylammonium fluoroborate (without irradiation). Photoaffinity label was added to Ringer's solution used to superfuse test node. Temperature: 11 °C.



light source and washing the fibre with Ringer's solution, about 65% irreversible inhibition of the potassium current remained (Fig. 1d). Control experiments suggested that this irreversible inhibition arises by a photoreaction of the molecules bound to a receptor in the potassium channels and not from the action of photolytic products of the reagent. A solution irradiated before its application to the test node strongly inhibited the potassium conductance, but this inhibition was completely reversible. Exposing the test node to ultraviolet light in the absence of photoaffinity label showed that in our experimental conditions ultraviolet light alone had practically no effect on potassium conductance but a small effect on sodium conductance (Fig. 3b). These observations are in perfect agreement with similar observations by Fox¹².

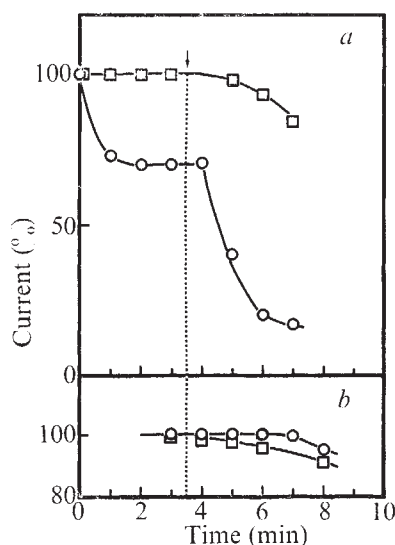


Fig. 3 Effects of photoaffinity label (a) and ultraviolet light (arrow) in the absence of photoaffinity label (b) on sodium ($\square$) and potassium ($\circ$) currents. Ringer's solution with 1 mM photoaffinity label was applied 3.5 min before irradiation. The small effect observed on the sodium system may be explained in terms of the effects of ultraviolet light *per se* in the absence of photoaffinity label. Temperature 11°C.

Photoaffinity label applied by diffusion from the cut ends of the fibre to the internal side of the nodal membrane caused, without irradiation, a similar selective inhibition of the potassium conductance (Fig. 1b). Surprisingly, irradiation with ultraviolet light had in this case the opposite effect to that in the case of external application of photoaffinity label (Fig. 1b). Instead of a further decrease there was an increase in potassium current, indicating that the binding site for the quaternary ammonium group is situated close to the internal opening of the potassium channel. According to this model, during irradiation in the case of application from the internal side of the axon, the 'reactive tail' of the photoaffinity label can react only with sites outside the channel—for example, with proteins from the axoplasm, the latter thereby being removed from the channel. When applied from the external side the label enters the channel far enough for a covalent attachment to sites inside the channel.

Analysis of the kinetics of potassium conductance activation after step depolarisation in the presence of photoaffinity label revealed a further difference between the internal and external site of the axonal membrane. When applied from the outside, without irradiation, the increase in potassium conductance was much slower compared with the control (Fig. 4a). After application from the inside the increase in potassium conductance was faster, and irradiation reduced it again to its original value (Fig. 4b). After the rapid increase in potassium conductance with some fibres a small inactivation of the potassium current was

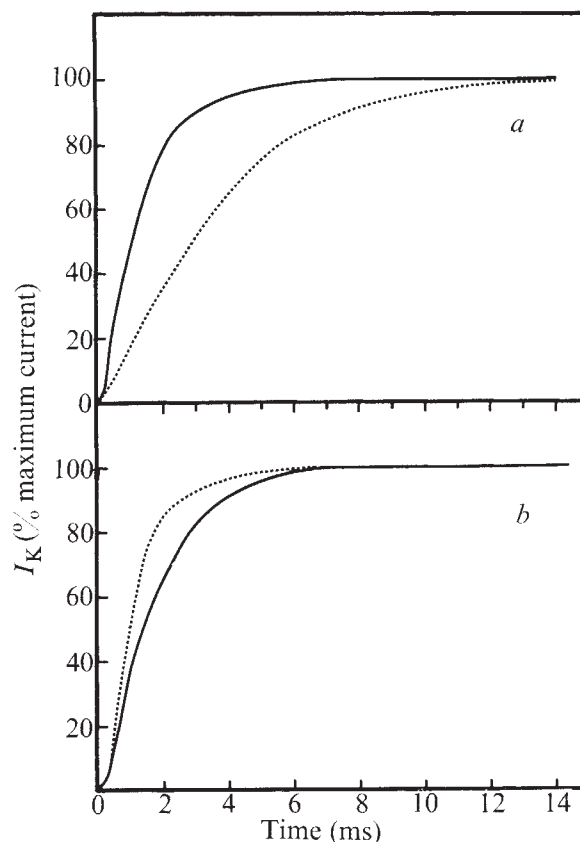


Fig. 4 Relative potassium current in the absence (solid line) or presence (dashed line) of photoaffinity label applied internally (b) or externally (a) without irradiation. a, 4 mM; b, 6.6 mM. Temperature, 11°C.

observed. This result is in agreement with experiments described by Armstrong^{13,14}, who observed an inactivation with internally applied benzyltriethylammonium and similar TEA derivatives.

One clear advantage of using our photoaffinity label is that its synthesis is straightforward, thus permitting radioactive labelling. Radioactive 4-azido-2-nitrobenzyltriethylammonium fluoroborate could be an important tracer for receptors associated with potassium channels.

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α Foetoprotein and serum albumin show sequence homology

α FOETOPROTEIN (AFP) is a major plasma protein of the foetus¹, where it is produced by the yolk sac and the liver². In adults its concentration is extremely low³ except in patients with tumours such as hepatomas and teratomas^{4,5}. AFP has a molecular weight of 70,000, consists of a single polypeptide chain and contains about 4% carbohydrate⁶. The similarity of the chemical properties of AFP and albumin and the fact that their concentrations in the serum are high and inversely related⁷ has led to the suggestion⁸ that AFP may be a foetal counterpart of albumin. We have determined amino acid sequences of fragments of human AFP and found homology between these and the albumin sequence.

were separated on Sephadex G-100 (Pharmacia) equilibrated with 6 M urea and 1 M propionic acid. Sequence determination was carried out on an automatic sequencer (Beckman, Model 890). The phenylthiohydantoin (PTH) amino acids were identified with gas chromatography and amino acid analysis following hydrolysis back to amino acids both in hydrochloric acid and hydriodic acid.

Cyanogen bromide cleaves AFP to seven peptides detectable by gel electrophoresis. Their molecular weights are 23,000, 12,000, 9,000, 8,000, 6,500, 4,000 and 3,500 (ref. 11). The 23,000 (I) and 12,000 peptides (II) were purified and their N-terminal sequences determined. Comparison of these sequences with the albumin sequence¹²⁻¹⁵ revealed significant homology between the AFP fragments and certain parts of the albumin molecule (Table 1). The percentage of same-sequence positions between the two peptides and human and bovine albumin was 37-45. Computer search for homology between the AFP fragments and known protein sequences¹⁶ revealed no further significantly homologous sequences and clearly established the significance of the similarity with albumin (Table 2).

These sequence data clearly indicate that AFP and albumin have a common ancestor. This is in line with

Table 1 Homology between fragments from AFP and albumin¹²⁻¹⁵

K N F G	T R T F Q A I T V	T K L S* Q K F T	(K)†	V (L)	F T Z I Q	AFP cyanogen bromide fragment I
Q K F G	E R A L K A W S V	A R L S Q K F P	K	A E	F V E V T	Albumin (bovine) 202-230
Q K F G	E R A F K A W A V	A R L S Q R F P	K	A E	F A E V S	Albumin (human) 203-231
S Y I C*	S Q Q D T L S N K I T E	S S K L T T	L E	R G Q S I	I	AFP cyanogen bromide fragment II
K Y I C	B B Z B T I S S K L K E	C K D P C L	L E	K S H C I	A	Albumin (bovine) 260-289
K Y I C	Z B Z B S I S S K L K E	C K E P C L	L E	K S H C I	A	Albumin (human) 261-290

*Assignment for serine as against cysteine should be regarded as extremely tentative due to the difficulty in distinguishing between the respective PTH derivatives using gas chromatography and back hydrolysis to amino acids.

† Tentative identification.

Human AFP was purified using immunoadsorbents⁹. The purity of AFP was checked with gel electrophoresis and immunological tests. The preparations used did not react with anti-normal human serum (Behringwerke) in immunodiffusion when tested at a concentration of 5 mg ml⁻¹. The procedures used and characterisation of the AFP preparations have been described in detail^{5,9}. AFP was reduced, alkylated with iodoacetamide and cleaved with cyanogen bromide¹⁰. Peptides resulting from the cleavage

their general similarity. Their chemical properties differ markedly only with respect to carbohydrate content. Albumin does not contain carbohydrate, whereas AFP is a glycoprotein⁶.

Our data do not permit accurate estimation of the degree of homology between AFP and albumin. The comparison is based only on 59 residues (out of about 600). Furthermore, the N-terminal sequences of albumin¹⁷ and AFP¹⁸ do not show homology. This may be due to insertion of additional

Table 2 Histograms of sequence comparison between AFP fragments and sequences in 732 other proteins*

Score	Peptide I	Homologous sequence	Peptide II	Homologous sequence
0	9,404		8,615	
1	16,411		16,551	
2	14,869		14,686	
3	8,658		8,565	
4	3,512		3,745	
5	1,163		1,258	
6	277		305	
7	95		85	
8	11		14	
9	0		2	Immunoglobulin K-chain-human Roy 172-201 Immunoglobulin μ -chain-human Ou 150-179
10	0		0	
11	0		1	Human albumin 261-290
12	0		1	Bovine albumin 260-289
13	2	Human albumin 203-231 Bovine albumin 202-230	0	

*Computer analysis carried out at the National Biomedical Research Foundation using existing sequence information¹⁶. The analysis compares the sequences of the AFP fragments with sequence fragments of corresponding length that are contained in the known sequences of 732 other proteins. The columns under peptides I and II indicate the number of sequence fragments with a given degree of similarity with the AFP fragments. This is expressed as the number of identical residue positions (=score) between the AFP sequence and the sequence with which it is compared.

sequence to the N-terminal of AFP or a deletion or post-synthetic modification in albumin. The fact that the pieces in the albumin sequence homologous with the relatively large AFP peptides are separated only by 30 amino acids, suggests rearrangement of these pieces in either one of the molecules.

Albumin binds a wide variety of different substances including fatty acids, drugs, and dyes¹⁹. AFP has been shown to bind certain hormones²⁰ and various dyes²¹.

These similarities and the sequence homology suggest that albumin and AFP have similar functions as general carriers in plasma and perhaps in the maintenance of the osmotic pressure. In addition, AFP probably has special functions offering an advantage over albumin to the foetus. This may be connected with immunosuppression²².

A detailed report on these results and further chemical characterisation of AFP will appear elsewhere.

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Quantitative adaptation of isoacceptor tRNAs to mRNA codons of alanine, glycine and serine

THE optimum efficiency of protein biosynthesis depends on the availability of aminoacyl-transfer RNAs (tRNAs) in the neighbourhood of active polysomes, among other factors. The intracellular tRNA level is directly proportional to the composition of the amino acids incorporated into proteins being synthesised¹. This continuous and selective adjustment takes place in prokaryotes and eukaryotes and in non-differentiated as well as in highly specialised cell systems². We report here that such a proportionality takes place at the molecular level between the frequency of synonymous codons in messenger RNAs (mRNAs) and the distribution of the corresponding isoacceptor tRNAs. A correlation was found for the alanine, glycine and serine codons from viral nucleic acids and eukaryotic iso-tRNA

sets fractionated on reversed-phase chromatography as well as for the preponderant tRNAs involved in the translation of fibroin mRNA in the posterior silk gland of the silkworm *Bombyx mori* L.

The overall ratio of different tRNAs must include a relationship between the frequency of synonymous codons in mRNAs being decoded and the respective set of iso-tRNA species. To verify this relationship, the distribution of synonymous codons for the predominant amino acids in mRNA and the anticodon distribution of the corresponding isoacceptor tRNAs must be known. The former can be obtained from a statistical study of a large number of mRNAs of known sequence in normal tissue or from the structural analysis of the main mRNA of a specialised tissue, whereas the latter is obtainable from the codon response of fractionated tRNA species and from sequences.

In this study the average frequency of synonymous codons in viral RNAs of which the sequence is partially or completely known is compared with the average distribution of the respective iso-tRNA species in several eukaryotes. According to the general analysis of Grantham³ with coliphage RNAs, the distribution of frequencies for degenerate codon positions is far from random. We assume that the same distribution frequency occurs in mRNAs from

Table 1 Average distribution of iso-tRNAs from eukaryotes and frequency of synonymous codons for alanine, glycine and serine in viral RNAs and DNA

	Codon response of iso-tRNA	Mean value $\pm$ s.d. (%)	Viral codons (%)	
Alanine	U		32	
	GCC	73.4 $\pm$ 4.6	21	82
	A		29	
Glycine	GCG	26.6 $\pm$ 4.6	18	18
	U		58	
	GG	65.8 $\pm$ 1.9	21	79
	C		9	
	A		12	21
Serine	GG	34.2 $\pm$ 1.9	8	
	G		12	
	U		26	
	AG	32.3 $\pm$ 5.4	14	57
	C		17	
	UCC	56.3 $\pm$ 2.4	23	23
	A			
	UCG	11.4 $\pm$ 4.3		

The proportions of iso-tRNA species are taken from mean values calculated from 15 independent elution profiles on reversed-phase chromatography for different eukaryotic cells. The codon response of each peak was measured by ribosome binding and the distribution obtained from the elution profile (for tRNA^{Ala}, see refs 4 and 5; for tRNA^{Gly}, ref. 4; and for tRNA^{Ser}, refs 4, 6 and 7). For iso-tRNA^{Ala} species chromatographic data are provided from *Drosophila*⁴, rat liver⁹, rabbit liver¹⁰, rabbit reticulocyte¹¹, calf muscle¹², human lymphoblasts¹³ and brain¹⁰. The minor iso-tRNA^{Ala} species not only decodes GCG, but also GCA; it seems to be a mixture of a third species recognising GCU and GCC in yeast⁵, as in prokaryotic organisms. A similar situation exists in the silkworm of *B. mori* L.³¹. For iso-tRNA^{Gly} species data were obtained from *Drosophila*⁴, rat liver⁹, rabbit reticulocyte¹¹, calf muscle and lens¹². For iso-tRNA^{Ser} species data come from *Drosophila*⁴, rat liver (refs 7 and 14, and H. Rogg, unpublished), rabbit liver¹⁰, rabbit reticulocytes¹¹, hen liver¹⁵, human lymphoblasts¹³, rat adrenal cortex, glioblastoma C-6 (H. Rogg, unpublished) and beef brain⁷. The frequency of alternative codons in partially and completely sequenced viral RNAs was determined from structural data compiled by Barrell and Clark¹⁶. Additional data were kindly provided by Fiers before publication of the total sequence of bacteriophage MS2 RNA coding for the A protein¹⁷ and the partial 3'-end sequence of MS2 RNA coding for viral RNA polymerase¹⁸, and by Guillely¹⁹ for the partial sequence of RNA from the tobacco mosaic virus coat protein cistron. Of 980 analysed codons, 35 comes from f2 RNA, 678 from MS2 (393 from A protein, 129 for coat protein and 156 for replicase), 32 from QB, 91 from R17 and 77 from TMV. We have also taken into account 67 codons from the G gene coding for spike protein of ϕ X174 DNA²⁰. The sampling for alanine, glycine and serine are respectively 82, 48 and 89.

Table 2 Distribution of iso-tRNAs, amino acid-tRNA ligases and codons for alanine, glycine and serine in the posterior silk gland of *B. mori* L. on day 8 of the fifth instar

	Alanine			Glycine			Serine			Refs
Amino acids of fibroin (%)	29			46			12			22
Amino acid-tRNA ligases (%)	31			42			13			29
tRNA (%)	26			42			16			24-26
Iso-tRNA (%)	3	6	17	20	7	15	5	9	2	27, 30
Anticodon (3'-5')*	(CGU)	(CGG)	CGI	CCG	(CCN)	CCU*	(UCG)	AGI	AGC	31-33
Codons of fibroin mRNA (5'-3')			GCU	GGU		GGA		UCA		23
Codons of fibroin mRNA (%)			29	27		19		12		—

*Presumed anticodon in parentheses.

†Estimated content according to the oligoribonucleotides analysis carried out by Suzuki and Brown²³ and to the amino acid composition of fibroin²². For calculating the linear correlation coefficient, we have taken both tRNA^{Ala} species able to recognise GCU codon.

highly differentiated organisms. The three tRNAs studied correspond to three main amino acids (alanine, glycine and serine) which account for $24 \pm 1\%$ of the residues in common protein. The base in the 5' position of the anticodon of their respective iso-tRNAs can be of four different kinds:

U
inosine can recognise XYC codons in tRNA^{Ala} and tRNA^{Ser},
A

guanosine recognises XY_C^U for tRNA^{Ala}, tRNA^{Gly} and

tRNA^{Ser}, uridine decodes GX_G^A in tRNA^{Ala} and tRNA^{Gly}

and cytidine UCG in tRNA^{Ser} (see ref. 4 for review).

Table 1 shows the results of a statistical analysis of the composition of three iso-tRNA sets from 15 eukaryotic cell types after fractionation by reversed-phase chromatography. We have only retained the quantitative variations of the concentration of each isoaccepting species and *a priori* ruled out qualitative or structural modifications detectable with this type of chromatography. In spite of differences in resolution and some inversions in elution order due to experimental conditions, the variability in the number and proportion of the iso-tRNA species is small. It is thus possible to assign average values.

Table 1 also shows the frequency of synonymous codons for alanine, glycine and serine, which represent 22.4% of 980 codons analysed from viral RNAs and DNA ϕ X174. Their distribution is non-random: XYU predominates. The correlation between the distribution of codons and the intracellular levels of the respective isoaccepting species corresponding to these codons is highly significant ($r=0.93$).

It is also possible to correlate the iso-tRNA levels and codons frequency of a predominant mRNA in a specialised tissue such as the posterior silk gland of *B. mori* L. The exponential accumulation of fibroin mRNA (mRNA_F) during the last larval instar up to 3.5% at the sixth day²¹ leads to a massive biosynthesis of fibroin accounting for at least 75% of the proteins synthesised at the end of the secretion phase. Alanine, glycine and serine represents 87% of the total amino acids of fibroin²². Isolation and partial structural analysis of the 3'-exoguanlyc oligoribonucleotides of mRNA_F enabled Suzuki and Brown²³ to deduce the main codons for fibroin: GCU for alanine, GGU and GGA for glycine in a 1.4 : 1 ratio and UCA for serine. The distribution of glycine codons is markedly different from that in viral nucleic acids, for which the GGU : GGA ratio is 6.5 (Table 1). This distribution is sufficiently different to stimulate a distinctive composition of iso-tRNA^{Gly} species in the posterior silk gland.

Several studies of the fibroin translational apparatus provide information about the intracellular levels of tRNAs during the fifth instar²⁴⁻²⁸, their cognate ligases²⁸⁻²⁹, the changes of iso-tRNA species^{27,30} and the nature of their

anticodons determined either by response to codons³¹ and to polynucleotides^{32,33}; or directly analysed by sequencing; or by their modified nucleoside content (J.-P. Garel, unpublished). Table 2 clearly shows that the posterior silk gland iso-tRNAs match the peculiar distribution of their cognate codons in fibroin mRNA ($r=0.99$). Note that the ratio between the two main iso-tRNA^{Gly} species (1.33) is different from that generally found (1.92, see Table 1).

The quantitative adaptation of iso-tRNA population to codon frequency of active mRNAs strongly suggests a coordinated regulation between the codon requirements of mRNA and the corresponding aminoacyl-tRNA species.

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Bifurcated hydrogen bonds and flip-flop conformation in a modified nucleic acid base, gc⁶Ade

FROM an analysis of X-ray diffraction data on modified bases of transfer RNA (tRNA) and their ribonucleosides, we have established that these molecules can be made to undergo, by suitable changes in pH, a reversible flip-flop conformational change from one stable state to another across a high potential barrier. We have also shown that positive charges adjacent to C-H bonds in the heterocyclic ring of nucleic acid bases polarise these bonds and enable them to take part in strong hydrogen bonds to appropriate acceptors.

The modified nucleoside *N*⁶-(*N*-glycylcarbonyl)adenosine (g⁶A), previously termed *N*-(purin-6-ylcarbonyl)glycine ribonucleoside¹, is an analogue of *N*⁶-(*N*-threonylcarbonyl) adenosine (t⁶A), and has been isolated² from enzyme digests of unfractionated yeast tRNA. We have carried out X-ray crystallographic studies of these anticodon-adjacent hypermodified bases at or near acidic (pH ~ 0) and alkaline (~ 8.0) pH, and of their ribonucleosides at pH ~ 3.5. At alkaline

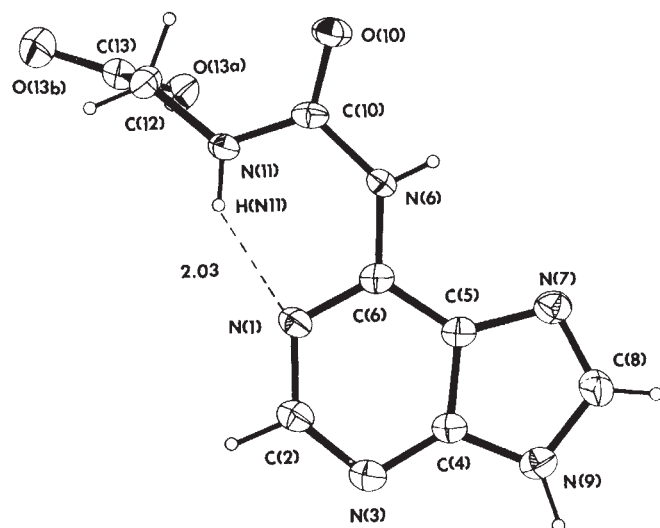


Fig. 1 Conformation of gc⁶Ade anion as found in the crystal structure of (gc⁶Ade)⁻K⁺. Note internal hydrogen bonding from H(N11) to N(1).

pH tc⁶Ade and gc⁶Ade bases are anions and these were crystallised (pH ~ 8.0) and studied by us as the corresponding potassium salts. The molecular structure of the gc⁶Ade anion as obtained from the crystal structure³ of (gc⁶Ade)⁻K⁺ displays two important features (Fig. 1); the base takes up the 'distal conformation'⁴ and an intramolecular hydrogen bond is formed from the hydrogen on N(glycine) to the N(1) of adenine. These

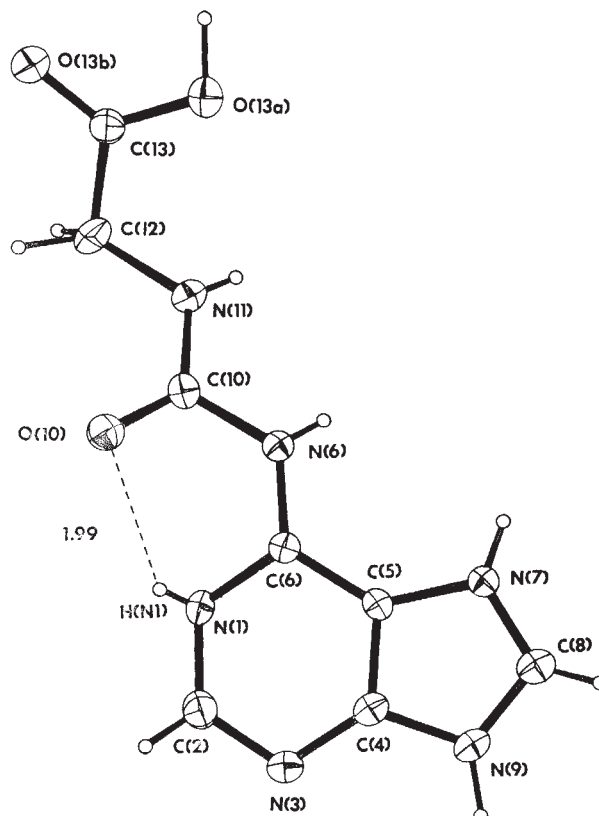


Fig. 2 Conformation of gc⁶Ade dication as found in the crystal structure of (gc⁶Ade)²⁺ (2Cl)²⁻. The protonation on N(1) has caused a change of conformation about the N(6)-C(10) bond giving rise to a different internal hydrogen bond, N(1)-H(N1) ... O(10).

two features are found in the structure of (tc⁶Ade)⁻K⁺·4H₂O (ref. 4) and also in the structures of the uncharged ribonucleosides t⁶A (ref. 5) and g⁶A (R.P., and G.B.C., unpublished). We discuss here the chemical implications of these two conformational features; their biological role has been suggested elsewhere⁴⁻⁵.

At acidic pH, the site of protonation for purine is N(1) although it has been argued⁶ that it is N(7) or N(9). Monoprotonated adenine derivatives such as adenine hydrochloride hemihydrate⁷, 3'-AMP (ref. 8), and 6-methyladenine hydrochloride⁹, all show protonation on N(1). In gc⁶Ade, N(1) is intramolecularly hydrogen bonded to H(N11) (Fig. 1); furthermore, the atoms N(11) and H(11) are held in this orientation by a series of conjugated covalent bonds, and are not free to move away from N(1). Consequently, it was of interest to determine in what conditions N(1) of gc⁶Ade can be protonated. We therefore treated gc⁶Ade with an excess of concentrated HCl solution to obtain crystals of the dihydrochloride derivative

Table 1 Table of bifurcated hydrogen bonds and weaker hydrogen interactions

D-H ... A ₁ *	H ... A ₁	D ... A ₁	D-H ... A ₁	A ₁ ... H ... A ₂
D-H ... A ₂	H ... A ₂	D ... A ₂	D-H ... A ₂	(sum)†
N(1)-H(N1) ... O(10)	1.99°A‡	2.616 Å	132.1°	91.8°
N(1)-H(N1) ... Cl(2 ⁱ)	2.49‡	3.141	136.1	360
N(7)-H(N7) ... O(13b ⁱⁱ)	2.22‡	2.847	153.5	84.3
N(7)-H(N7) ... Cl(2 ⁱⁱⁱ)	2.78	3.185	121.8	360
C(2)-H(C2) ... Cl(1 ⁱ)	2.93	3.649	140.6	86.5
C(2)-H(C2) ... Cl(2 ⁱ)	2.78	3.337	125.8	353
C(8)-H(C8) ... Cl(1 ⁱ)	2.51‡	3.349	150.3	93.9
C(8)-H(C8) ... Cl(2 ⁱⁱⁱ)	2.88	3.260	108.7	353

*D is the donor and A is a possible acceptor atom.

†Sum = angle (D-H ... A₁ + D-H ... A₂ + A₁ ... H ... A₂).

‡(i) 1-x, -y, 1-z; (ii) -x, ½+y, -½+z; (iii) 1-x, 1-y, 1-z.

‡Considered as hydrogen bonds; the other contacts are considered as weaker hydrogen interactions.

of gc^6Ade . These crystals are monoclinic, space group $\text{P2}_1/\text{c}$ with unit cell parameters: $a = 13.069(3) \text{ \AA}$, $b = 9.878(1) \text{ \AA}$, $c = 12.866(4) \text{ \AA}$, $\beta = 134.27(1)^\circ$, $D_{\text{obs}} = 1.65 \text{ g cm}^{-3}$, D_{calc} (for $\text{C}_8\text{H}_{10}\text{N}_6\text{O}_3\text{Cl}_2$) = 1.62 g cm^{-3} , $Z = 4$, temperature = $22 \pm 3^\circ\text{C}$, $\lambda(\text{CuK}\alpha) = 1.54051 \text{ \AA}$. Using diffractometer data (2,582 reflections to the limit $2\theta = 160^\circ$ for $\text{CuK}\alpha$) the crystal structure was solved, and refined by the least-squares method to $R = 0.056$. Detailed correction for absorption was carried out. The hydrogen atoms were located from electron-density difference maps and their parameters refined by the least-squares method.

The structure analysis shows (Fig. 2) that the molecule is a dication with protonation on both N(1) and N(7) or N(9). Concomitant with the protonation is a change in the conformation of the molecule, a *cis-trans* isomerisation across the N(6)–C(10) bond stabilised by the formation of an intramolecular hydrogen bond N(1)–H(1) $\cdots$ O(10). Thus, the change in pH to the acidic side 'flipped' the molecule from one stable conformation to another stable one across a high potential barrier; the original conformation can be restored by increasing the pH. The basic pK_a of gc^6Ade measured by us is 2.6, compared with a pK_a of 3.8 for adenine. It seems that the hydrogen bonding causes an hysteresis effect; the titration curve in one direction is not reproduced on back titration, as in the cytosine moiety of DNA¹⁰. The double protonation of the adenine ring has caused considerable delocalisation of the charge in the adenine ring, and has highly polarised^{7,11} the N(1)–H, N(7)–H, C(2)–H and C(8)–H bonds so that they take part in bifurcated hydrogen bonds—or weaker bifurcated hydrogen interactions (Table 1). The C(2)–H and C(8)–H hydrogens have no such contacts in the neutral and anionic forms of gc^6Ade . In all these bifurcated bonds and weaker bifurcated interactions, the hydrogen atom lies close to the plane through the donor and the two acceptor atoms^{12,13}.

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Balance between swelling pressure and collagen tension in normal and degenerate cartilage

ARTICULAR cartilage contains a high concentration of acid glycosaminoglycans (GAG), reaching 6% by wet weight and associated with fixed charge densities up to 0.2 mEq g^{-1} . This leads to considerable swelling pressure within cartilage, due to, first, the strongly non-ideal osmotic pressure, character-

istic of polymer solutions, which increases sharply with concentration and, second, the ionic contribution, in accordance with the Gibbs–Donnan equilibrium. Ogston and Wells^{1–3} have estimated the values of these two components and I have calculated them from my experimental data on cartilage^{4,5}. The two components of swelling pressure are approximately of the same order of magnitude and can reach values as high as 1.7 kg cm^{-2} (refs 4 and 5). Since normal cartilage does not swell in solution, even when it is removed from the joint and cut into thin ($250 \mu\text{m}$) slices (lowest curve, Fig. 1), this implies that its high swelling pressure must be counteracted by considerable elastic forces within the collagen fibre network. It has been known for some time that the concentration of GAG gradually increases from the articular surface to the deep zone (for example, refs 6 and 7). A typical variation of total GAG content with depth, measured as fixed charge density, is shown in Table 1. I now suggest that this particular profile is adapted to the physiological function and mechanical properties of cartilage.

Table 1 Ionic components of swelling pressure corresponding to different values of fixed charge density

Distance below articular surface (mm)	*Fixed charge density (mEq per g of wet tissue)	†Ionic component of swelling pressure (kg cm^{-2})
0	0.05	0.16
0.25	0.10	0.45
0.75	0.15	0.8
1.75	0.18	1.7
2.25	0.20	2.1

*The figure for the surface concentration of charged groups was obtained by X-ray probe analysis¹⁷. The other figures were obtained by the tracer cation method¹¹ and verified by chemical analysis⁵.

†The values of the ionic component of swelling pressure were calculated using Na^+ and Cl^- partition data obtained experimentally for slices of human femoral head cartilage (my work with W. D. S. Freeman, in preparation), and a formula given by Katchalsky *et al.*¹⁸, to estimate the fraction of osmotically 'active' counterions. Similar values were obtained when a procedure suggested by Wells¹² and based on Manning's approach²⁰ was used in the calculations.

First, it must be remembered that a high GAG content is essential to the load-bearing requirements of joints for it confers on the tissue low hydraulic permeability⁸ and high swelling pressure¹. It thus serves both to limit the amount of liquid lost when cartilage is loaded in compression (and hence the resulting creep), and to ensure the speedy 'recovery' of cartilage during the 'off-load' intervals in the walking cycle^{4–6}.

If, however, the GAG concentration were uniformly high throughout the cartilage thickness, right up to the interface with synovial fluid, there would be a steep osmotic pressure gradient across this interface. The local tensile stresses in the collagen fibre network oppose the local swelling pressure gradients at any point within the tissue, and so, at the surface, the collagen fibres would have to be under exceptionally high tension to oppose the very high osmotic pressure gradient which would be present there. In reality, the GAG concentration at the surface is at its lowest, rising to a broad peak in the middle zone. Table 1 shows the values of the ionic component of swelling pressure as a function of distance from the surface. A fairly even gradient of swelling pressure is observed in the tissue, with only a small fraction of the maximum pressure at the interface with synovial fluid. This grading of stresses, however, seems to be designed more as a protection against long term fatigue, since collagen fibres in normal cartilage do not disrupt instantaneously or allow any measurable swelling when thin slices from whatever zone are exposed to solution (as mentioned above and shown in Fig. 1, lowest curve).

In contrast, degenerate cartilage swells easily. It has long

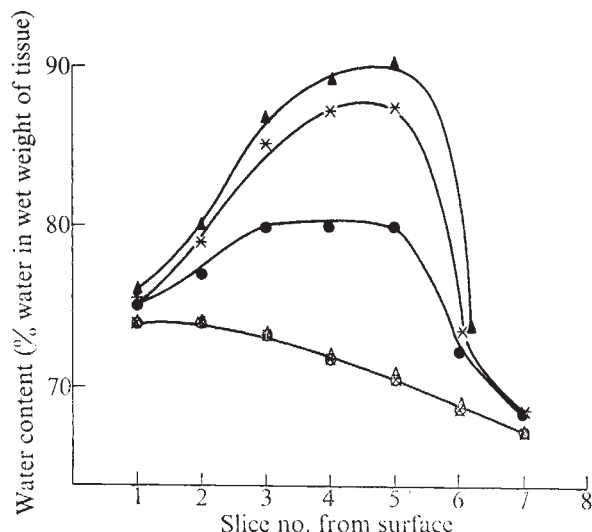


Fig. 1 Hydration in normal and degenerate cartilage. The moisture distribution in the chunk was obtained by slicing the latter on a freezing microtome into 250- μ m slices and weighing these directly. The subsequent swelling of the slices was determined by immersion in appropriate solution and weighing again after equilibration. Degenerate cartilage: ●, as cut; ▲, after soaking in 0.015 M NaCl; *, after soaking in 0.15 M NaCl. Normal cartilage: ○, as cut; △, after soaking in 0.015 M NaCl; ×, after soaking in 0.15 M NaCl.

been known that one of the symptoms of cartilage degeneration is increased water content (for example, refs 9–11). This may seem surprising, for cartilage degeneration is usually accompanied by a loss of glycosaminoglycans. Few plausible explanations have been suggested for this paradox. I have suggested¹² that a failure in the elastic restraint of the collagen network enabled fibrillated tissue to swell to a higher degree of hydration in spite of the relatively low osmotic pressure of its decreased GAG content. I now present data in support of this hypothesis.

Figure 1 shows a profile of variation in water content with distance from the articular surface for both normal and mildly degenerate cartilage (my work with M. Venn, submitted for publication). In the normal cartilage, the water content seems to be little affected by the local GAG content. In degenerate specimens there is a distinctly different pattern, with a maximum in the middle zone, which I interpret as follows. If the collagen network is partly damaged, its restraining effect on the swelling will be diminished and the hydration profile in cartilage should parallel its GAG content (which is known, in mildly degenerate tissue, to be rather low at the surface but still reasonably high in the middle zone (refs 11 and 12)). An intact collagen network is often still preserved in the deepest zone and thus there is no possibility of swelling in this region.

I further found that when a full depth chunk of surface-fibrillated cartilage is cut into 250- μ m parallel slices, and these are immersed in buffered physiological saline (0.15 M NaCl), considerable swelling occurs in the slices from the middle zone, compared with their original water content (Fig. 1). This is consistent with the increased osmotic stress in these slices when exposed directly to isotonic saline solution, compared with the situation within the chunk. The swelling of middle zone slices was even more pronounced when they were immersed in a hypotonic solution (0.015 M NaCl), since the ionic component of the pressure differential was thus further increased (Fig. 1).

These results point to the fact that increased hydration in degenerate cartilage is due to damage of the collagen network and not, as suggested¹³, to an increased fraction of so-called "bound water".

Finally, I wish to draw attention to a further implication of the above findings. *In vivo* the loss of GAG from the surface in an early stage of degeneration must, by this reasoning, lead to a steeper swelling pressure gradient in the middle zone, which should in turn lead to a greater stress on the collagen network there. It seems a reasonable supposition that fibres under tension are more liable to scission, whether by mechanical or enzymatic agents. I therefore suggest that once the surface layer of cartilage begins to lose its GAG, a self-perpetuating destructive process ensues in which successively deeper layers are exposed to gradual collagen disruption through GAG depletion.

It is still not known whether the primary event which leads to cartilage degeneration is glycosaminoglycan loss brought about enzymatically, with the damage to the collagen network following¹⁴, or whether mechanical fatigue¹⁵ is the direct cause of fibrillation, with the damaged collagen network subsequently enabling the proteoglycans to escape from the articular surface. But the observation that both in the early stages of an experimentally induced osteoarthritis in the dog¹⁶, and in some visually intact specimens of human osteoarthrotic cartilage removed for joint replacement (my work with M. Venn, submitted for publication), the water content is invariably increased, while the GAG levels remain normal, suggests (because of the casual association put forward here between the swelling of cartilage and a damaged collagen fibre network) that collagen fatigue and breakdown may be the earliest in a destructive chain of events.

Whatever the nature of the first step, it is obvious that early swelling of cartilage must also lead, through dilution of GAG in the tissue, to increased hydraulic permeability and decreased osmotic pressure⁴, and thus must create an unfavourable mechanical environment in subsequent stages of the self-destructive process outlined here.

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Growth of algal symbionts in regenerating hydra

BETWEEN 10 and 20 photosynthetic algae are maintained per digestive cell in green hydra, the freshwater polyp. In normal conditions the number of symbionts maintained within the host remains stable—the algae do not overgrow

the host nor do the animals outgrow the algae¹. In experiments described here we found that when the upper two-thirds of a hydra was removed, there was a pronounced increase in the number of algae per digestive cell in the remaining peduncle of the animal. The observed increase in algal numbers was not a function of starvation and could be abolished by grafting a head, from a donor animal, on to the peduncle. We believe that our experiments are the first to show that the reproduction of symbiotic algae may be influenced by the host and that the presence of a head, thought to be responsible for maintenance of polarity and other morphological phenomena in hydra², may also influence the reproduction of symbiotic algae.

We used a variant of the green hydra, referred to as the English strain. Animals were reared and maintained under a 12 h light/12 h dark photoperiod and fed *Artemia nauplii*³. Five experimental organisms were macerated together and a sample of the resulting cell suspension was examined under a compound microscope. The algae in 150 randomly selected digestive cells were counted^{1,4}.

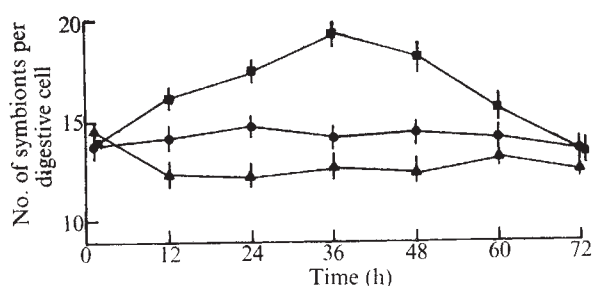
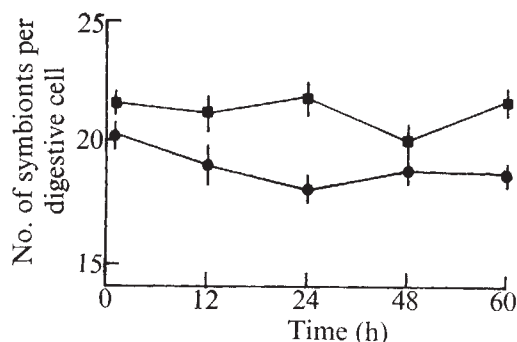


Fig. 1 Average number of algae per digestive cell from peduncle region of hydra at various times after removal of the upper two-thirds of the animal. Vertical bars show the standard error, $n = 150$ digestive cells. ■, Regenerating peduncle: analysis of variance indicates significant difference between points ($P < 0.001$). ▲, Peduncles removed from unfed animals at time of counting: analysis of variance indicates no significant difference between points ($P > 0.10$). ●, Peduncles with grafted heads: analysis of variance indicates no significant difference between points.

In our first experiment we removed the upper two-thirds (head, gastric and budding regions) of a group of hydra. At 12-h intervals after amputation, the algae per digestive cell were counted in the regenerating peduncles. Green hydra normally maintains about 15 algae per digestive cell in the peduncle region. The regenerating peduncles did not eat throughout the experiment. Thus for controls, peduncles freshly isolated from unfed, intact animals were also analysed. Figure 1 shows that in regenerating peduncles the number of algal symbionts per digestive cell increased 25% from about 14 to 20 within 36 h of amputation of the upper

Fig. 2 Average number of algae per digestive cell from gastric region of hydra. Vertical bars show the standard error, $n = 150$ digestive cells. ■, Gastric region from animals after amputation of peduncle: analysis of variance indicates no significant difference between points. ●, Number of algae per digestive cell of gastric region removed from unfed animals at time of counting: analysis of variance indicates no significant difference between points.



two-thirds of the animal. After this increase in symbionts there was a decline in numbers per cell until the original level was established at 72 h, when the peduncle had regenerated the head and could feed. No change in algae per cell was observed in the control animals (Fig. 1b).

To determine the possible influence of the head region on the growth of algae in the peduncle, we grafted heads from donor animals to the amputated peduncles. As before, these organisms were not fed and were analysed every 12 h. As Fig. 1 shows, there was no increase in the number of algae per digestive cell in peduncles with grafted heads. Furthermore, there was no change in the number of algae per digestive cell in the upper gastric region (with heads) after amputation of peduncles (Fig. 2). These cells in the upper region normally contain approximately 20 symbionts.

Apparently the presence of a grafted head region prevented or inhibited the algal increase observed in the absence of the head region. It has been suggested that the head region is the site of morphogenetic substances which maintain the normal polarity and organisation of the animal⁵. Our results suggest that factors associated with the head inhibit algal reproduction in the peduncle also. These factors might act directly on the reproductive processes of the algae or indirectly through processes in the host's digestive cells in which the symbionts reside. When amputated, the peduncle tissue is removed from the influence of the head and the symbionts, now released from inhibition, begin multiplying.

It is not clear how the number of algal symbionts is reduced after the initial increase (Fig. 1). A possibility is that subsequent host cell division causes parcelling of the symbionts among the daughter digestive cells. The relationship between the head and animal cell division in the peduncle is unknown.

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³ Lenhoff, H., and Brown, R., *Natn. Cancer Inst. Monogr.*, **31**, 709-737 (1969).

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⁵ Wolpert, L., Hornbruch, A., and Clarke, M. R. B., *Am. Zool.*, **14**, 647-663 (1974).

Errata

In the article "Involvement of DNA in resistance of potatoes to invasion by *Phytophthora infestans*" by M. Yamamoto and K. Matsuo (*Nature*, **259**, 63; 1976) the legends to Figs 1 and 2 were transposed.

In the article "The complete nucleotide sequence of bacteriophage MS2 DNA: primary and secondary structure of the replicase gene" by W. Fiers *et al.* (*Nature*, **260**, 500; 1976) Figs 1 and 5 have been transposed.

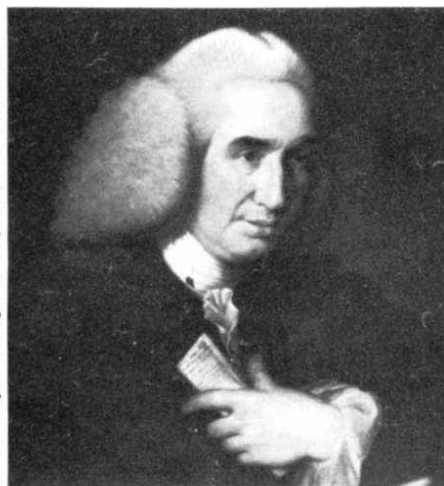
In the article "Brain immunoreactive gonadotropin-releasing hormone in Huntington's chorea and in non-choreic subjects" by E. D. Bird, S. A. Chiappa and G. Fink (*Nature*, **260**, 536; 1976) page 538, lines 8-9 In the female this could not account . . . should read In the female this could account. . . .

reviews

Enlightening chemistry

R. V. Jones

Photo: Royal College of Physicians of Edinburgh



William Cullen (1710–90), from the original by William Cochrane

THE Scottish intellectual enlightenment in the eighteenth century is rightly attracting attention among current historians of science in the US. A few months ago we had from Richard Olson an interesting book on *Scottish Philosophy and British Physics 1750–1880* (*Nature*, **258**, 285; 1975) on the effects of the Scottish common sense tradition on the development of British physics. And now we have from A. L. Donovan of the University of West Virginia a study of the interaction of two great Scots chemists of the enlightenment, William Cullen (1710–1790) and his pupil Joseph Black (1728–1799).

Donovan gives a good picture of the intellectual state of Scotland and its universities in the eighteenth century, and one of his aims is to demonstrate that "the advancement of scientific

Philosophical Chemistry in the Scottish Enlightenment: The Doctrines and Discoveries of William Cullen and Joseph Black. By A. L. Donovan. Pp. x+343. (Edinburgh University: Edinburgh, January 1976). £7.

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knowledge was a matter of both philosophical and practical concern in eighteenth century Scotland". He was drawn to study the question of how Black came to discover 'fixed air' (carbon dioxide) and this in turn led him to investigate William Cullen, with whom Black worked in Glasgow after graduating in arts and medicine. This was at about the time when, thanks largely to Cullen, chemistry was becoming a university study in its own right instead of being merely part of natural philosophy on the one side or medicine on the other.

If anything, Black was more of a natural philosopher, as shown by his work on specific heat and latent heat; but it was the medical need to find a solvent for renal stones that led him to recognise that chalk contained 'fixed air' which could be released as a gas by heat or acids, but which could also be transferred from one substance to another in solution without being released as effervescence. Donovan has gone to much scholarly trouble to trace this development in Black's mind—perhaps all the more interesting because it occurred against the background of



Joseph Black (1728–99)
by David Martin

Black's acceptance of the Phlogiston theory which Lavoisier's work later forced him to abandon.

Black's influence, of course, extended beyond chemistry, and the last chapter of the book surveys his interaction and friendship with John Robison and James Watt, to whom Black's concept of latent heat was especially relevant. And when Watt invented the condensing steam engine Black (who in later life weighed every golden guinea that his students paid him in fees to check that they had not been cheating by filing the coins) lent him one thousand pounds to develop the engine.

Donovan has produced a useful, detailed, and well annotated study of an important phase of the Scottish scientific heritage. □

SURELY the function of a book is communication between the author and reader. In this respect far too many contemporary scientific texts are deficient. They seem to operate on the assumption that the message is there—if you look hard enough. Aggregates of papers each by a different author with little editing or editorial comment are particularly prone to this elitism. It is then a happy event to find this book. It arose out of the course in advanced immunology sponsored by the Department of Immunology at the Royal Postgraduate Medical School and contains an amplification of the lecture notes. There has, however, been much

Immunology in vogue

A. J. S. Davies

The Immune System: A Course on the Molecular and Cellular Basis of Immunity. By M. J. Hobart and Ian McConnell. Pp. xxiii+357. (Blackwell Scientific: Oxford and London; Lippincott: Philadelphia and Toronto, 1975.) £5.00

useful editorial grading and cross-linking.

There are four major sections, each

containing several chapters, on immunochemistry, immunobiology, immunogenetics and immunopathology. Each section has a chatty editorial introduction by no means as prolix as their designation 'preambles' would suggest. The range of topics is considerable although by no means comprehensive. In the immunopathology section for example, there are chapters on the so-called tumour immunology, immunodeficiency diseases, and auto-allergic conditions but nothing on parasitic disease.

The illustrations, with the exception of one or two rather indeterminate electron micrographs (for example, on

p60) are first class and provide an important visual element to the verbal complexities of contemporary immunology.

Following the present vogue, the book is predominantly concerned with cells and molecules and, with the exception of the illustrations of T and B areas on p210, there is hardly any histopathology. The approach of the synthesist in immunology is long overdue, and although there is a good chapter on the structure and function of the lymphoid system by McConnell this is not enough for my taste. A section on immunopathology can

hardly be complete with no consideration of reactive changes in lymphoid organs. The book as a whole is so nearly comprehensive as to make consideration of remedy of these minor deficiencies worthwhile.

The book throughout is very readable, and the intention of the editors to communicate with their readers is made clear and is resoundingly successful.

In an age when new books tend to be greeted with a groan by overloaded minds here is one for your personal perusal. Don't leave it to the library—get one yourself, it's a splendid book. □

Methodical investigation of dynamic systems

Introduction to Mathematical Control Theory. (Oxford Applied Mathematics and Computing Science Series.) By Stephen Barnett. Pp. viii+264. (Clarendon; Oxford; Oxford University: London, 1975.) £5.75 net.

THERE has recently been a shift of interest in control theory from engineering towards applied mathematics. Although this reflects growing awareness of the range and depth of mathematical problems which have been formulated in terms of modern control theory it also sadly reflects a growing disillusionment on the part of the professional engineer at the failure of control theory to realise but a small fraction of what was promised a decade ago. In recent years there has been a significant return to linear system concepts to formalise the techniques necessary for analysing systems in which the numbers of control variables are large. This has led naturally to the use of state vectors and matrix system operators to describe the dynamic performance of systems. It is here that the first difficulties arise since not only are matrix equations often difficult to manipulate, but they do not provide a physical insight into the nature of the control problems which the semigraphical older methods of control theory provided.

There clearly is a need for basic textbooks which can introduce the newer aspects of control theory and, if possible, link them with more established techniques. The audience would include applied mathematicians new to the control field, as well as students and practicing engineers. Stephen Barnett's book roughly supplies this need. Engineers will not find it an easy book to read since matrix algebra at this level

is a demanding discipline. This is not made easier by the concise treatment given to many theorems but this is unavoidable in a work of this length. The reader will be struck, however, by the symmetry and compactness of the general theory which is beginning to evolve. The mathematician will find unusual outlets for studies in stability theory and the calculus of variations.

Although the book is by no means exhaustive in its range, it does cover a basic related set of topics and prepares the ground for further study. There are numerous worked examples and exercises but, as in most control textbooks, these describe problems which are almost trivial from an engineering point of view. It is argued that once the concepts have been mastered the extension to worthwhile problems is straightforward and is considerably aided by computational methods. It is of course here that the rift between theory and practice occurs. The formulation of real problems in terms of linear dynamic models, or often in any mathematical form, is difficult if not actually meaningless. Solutions, except in terms of empirical effort, are expensive to obtain and may then be impractical to realise. In spite of all this, sensibly applied control theory has much to offer in providing a framework for the methodical investigation of dynamic systems. This small but scholarly book will help to build such a framework.

J. M. Nightingale

Showing the flag

Geodynamics Today: A Review of the Earth's Dynamic Processes. (Sponsored by the British National Committee for Geodynamics for the Inter-Union Commission on Geodynamics.) Pp. 197. (The Royal Society: London, 1975.) £2.75 UK; £2.85 Overseas, including postage.

THE Geodynamics Project (1972–1977) is a loosely-coordinated international program of research into the dynamics and dynamic history of the Earth with emphasis on phenomena affecting surface and near-surface processes and structures. In 1973 the US Geodynamics Committee published, at no charge, a highly regarded and more or less definitive 235-page report on the scope and objectives of the Project. Soon after it appeared, the British National Committee for Geodynamics decided that something more than a preliminary British statement (published by the Royal Society in 1971) would be appropriate; and the result, published more than halfway through the Project, is *Geodynamics Today*. It is a sad reflection that in inflation-ridden 1970s Britain one has to pay to view the flag.

And the flag is a bit tatty at that. In this loosely-coordinated non-international program of 23 short state-of-the-art reviews on geodynamics some authors have dealt in generalities, others have resorted to detail; some have emphasised the past-to-present, others have concentrated on the present-to-future; some have included references and/or diagrams, others have gone for words only; some have put their heart into it, others have not. The result is a hotchpotch in which, the overriding geodynamic theme apart, subjects are linked only by virtue of the active or passive interest of the British scientists concerned. Nevertheless, the topics cover a wide range of geodynamic problems; and if no-one will wish to read everything, there is something here for almost everyone.

It is impossible to mention every subject covered and difficult to select a few. But of particular interest, because they are concerned with expanding themes of general consequence, are J. Sutton on the geodynamics of the Precambrian, E. R. Oxburgh on plate collisions past and present, I. G. Gass and others on the origin and emplacement of ophiolites, W. S. Fyfe on the influence of the hydrosphere on magma and D. H. Tarling on pre-Mesozoic palaeomagnetism. Also worthy of note, but for a different reason, is the article by N. N. Ambraseys, one of the few people to recognise the importance of historical seismicity studies, especially in the scientifically unpopular Mediterranean region.

If one general point emerges from this collection, or part of it, it is that British Earth scientists of various persuasions are particularly well placed, by inclination and experience, to lead the assault on the problems of pre-Wege-nerian geodynamics. It is to be hoped that the money will be available for them to do so.

Peter J. Smith

Lunar Mineralogy. By Judith W. Frondel. Pp. x+323. (Wiley-Interscience: New York and London, October 1975.) £11.20.

THE ending of the Apollo and Luna missions marks a significant turning point in the development of lunar science. Over the past five years efforts have concentrated on frantic reconnaissance and sample analysis in preparation for future missions, but now the pace has changed and problem-orientated studies and a more thoughtful evaluation of published data seem to be the most obvious of immediate future developments. In this situation the appearance of a book of this type is particularly valuable. About 850 pounds of rock were recovered in the Apollo programme, but it is important to remember that of this only about 10% has been extensively examined; approximately 20,000 individually identified samples remain available for future study.

This book has grown from two editions of a *Glossary of Lunar Materials* which the author prepared for distribution by NASA to principal investigators of the Apollo programme. It is arranged in the manner of Dana's *System of Mineralogy* and summarises all lunar mineralogical data published up to and including the Fifth Lunar Science Conference in March 1974. The author's task has been aided by the Earth's satellite, for rich repositories of rare and spectacular minerals such as pegmatites, true granites and hydrous vein deposits are conspicuously absent in the Moon; the rocks remain in a highly reduced state and there is a marked depletion in elements more volatile than iron. It is not surprising therefore to learn that only 60 valid and 14 tentatively identified mineral phases have so far been reported compared with about 2,200 on Earth; and there are no lunar diamonds.

Individual minerals are described in eleven systematic chapters; the treatment here is concise but adequate, and each section gives a good account of how particular minerals occur in lunar rocks. There are useful tables with selections of mineral analyses, photomicrographs wherever possible, and abbreviated references. The wisdom of including here abbreviated optical and X-ray data on minerals such as olivines is questionable; the photomicrographs and specimen photographs are of a high quality, and the eye is captivated by the beautiful scanning electron microscope photographs of iron crystals deposited in vugs. The systematic chapters are preceded by an excellent 16-page introduc-

tion which summarises the history and petrology of the lunar surface; this section can be thoroughly recommended to amateurs. With these in mind, however, it is disturbing to read on page 7 that "Total and partial melting, a relatively insignificant feature on Earth, is important in the petrogenesis of the lunar highlands".

The author and publishers are to be congratulated on the high standard of production. The book will be a standard reference for many years to come, and will be particularly valuable for those who have not worked on lunar minerals. The main text is supported by a bibliography, simple index and appendices which list the samples mentioned in the text and explain the sample-numbering system.

I. D. Muir

Lunar science

Lunar Science: A Post-Apollo View—Scientific Results and Insights from the Lunar Samples. By S. R. Taylor. Pp. xix+372. (Pergamon: New York and Oxford, 1975.) \$16.50; £6.90.

THE technological *tour-de-force* in landing men on the Moon in the six missions Apollo 11, 12, 14, 15, 16 and 17 and the drama of Apollo 13—in particular the extreme sophistication of the systems control—has perhaps overshadowed the scientific achievements in the study of the returned lava, breccia and soil samples and the observations from experimental apparatus, some still functioning on the Moon. How appropriate it would be in this bicentennial year of the US if NASA produced a history of this unprecedented scientific project: by what alchemy did imaginative planning emerge from committees of scientists and administrators in Washington. But in some ways the very triumph of the scientific collaboration has been buried in vast conference proceedings; and it has been left to Dr Ross Taylor to give a most admirable answer to the question insistently asked "What really did this project discover about the Moon?"

Many of the conclusions reached or reinforced by evidence brought back by the Apollo project are set out clearly by Dr Taylor, and the book is a remark-

able one: no scientist could fail to be fascinated and even those most learned about the Moon will learn something more. One miracle, however, was not achieved by the planners of the Apollo project: in spite of all attempts it has not entirely succeeded in bringing about an easy communication between petrologists and geochemists on the one hand and geophysicists on the other. In the many lunar conferences once the former begins talking about ferroperovskite and hedenbergite—ferrohedenbergite, the eyes of the latter become glazed; and when the latter begin speaking of electromagnetic transfer functions and magnetic Reynolds numbers, the conference room empties noticeably. In his treatment of the physics of the Moon, mainly in chapter 6 (only 29 pages), Dr Taylor falls from the high standard elsewhere.

Yet it was in this field that three of the most surprising and unexpected discoveries were made—positive anomalies in gravitational field occurring over the circular maria, remanent magnetisation of the returned specimens, along with magnetic anomalies in the lunar crust and moonquakes occurring predominantly at apogee and perigee. Dr Taylor does not treat these discoveries—fundamental to any understanding of the Moon's interior and its evolution—in the depth they deserve. For example in lunar magnetism he relies on a review, which he says provides "appropriate skeptical comments on current theories." Although some ideas which have been suggested to explain this unexpected finding are plausible—Velikovsky's prediction that the lunar surface was magnetised in 3,000 BC—some theories merit serious consideration. But some inconclusiveness is only to be expected when a new phenomenon is discovered. Observations that can be immediately understood in terms of currently accepted orthodoxy are not of so much interest as those which open up new approaches.

The fundamental significance of the non-hydrostatic figure of the Moon is ignored and thermal convection occurring by solid-state creep in its interior is not mentioned. The essential requirement of a theory of mascons—that is, that extra mass, whether lava or not, must be brought by lateral transport into the circular mare area—is not made clear. The implications of the remarkable correlation between the occurrence of moonquakes and the tides, and the analogous effect with regard to the enigmatic lunar transient events, are also not brought out.

S. K. Runcorn

obituary

Professor W. Ehrenberg, who died on November 19, 1975 at the age of 74, was born and brought up in Berlin. When a boy, he fell a victim to poliomyelitis which left him severely handicapped, but he never allowed this to impede his work in experimental physics, to which he transferred his main interest immediately after gaining his Ph.D. from Heidelberg in 1924. In 1926 he published with H. Mark and G. von Susich some now classic papers in which the natural width of the K X-ray lines was demonstrated and the two-crystal X-ray spectrometer proposed and analysed. With Ewald he conducted an experimental verification of the predictions of the latter's dynamical theory of X-ray diffraction. This and other X-ray experiments were carried out first in Berlin and later at the Technische Hochschule in Stuttgart, where he also made contributions on the diffraction of low-energy electrons and on the wave-mechanical theory of electrical contacts.

In 1933, with other refugees from the Nazis, he joined Professor Blackett's group at Birkbeck College, University of London, where he published papers on cosmic rays and neutron physics. Between 1936 and 1945 Ehrenberg worked at the EMI research laboratories, mainly on problems concerned with radar and the development of television tubes. Although possibly not so fruitful from a scientific point of view, this period of industrial research nevertheless gave him wide experience of electron beam devices and related techniques, which were to prove of considerable value in his later work. During this period he privately embarked on new studies; a paper on entropy and irreversible processes dates from this time, and thermodynamics became an abiding interest.

In 1945 Ehrenberg returned to Birkbeck as one of Professor Bernal's six

Nuffield research fellows: his rôle was to apply electron optical and X-ray optical techniques to problems of crystallographic instrumentation, while remaining free to supervise research in other fields. During the next decade he built up a flourishing research group at Birkbeck College. For him and his students it was a period of intense and exciting activity. In 1949 he published, with R. E. Siday, a theoretical paper on electron optics in which they reached the surprising conclusion that electron interference can be affected by the presence of a magnetic flux even if the electron paths do not pass through a magnetic field. This idea was independently put forward ten years later by Aharonov and Bohm and was then verified experimentally by R. G. Chambers.

On the experimental side he contributed during these years to X-ray optics, particularly to the focussing of X-rays by total reflection at glancing angle from slightly curved surfaces, a development from his experiments in Berlin some 20 years before; but he found the usefulness of the technique to be limited by the excessively stringent requirements for smoothness, a difficulty that has recently been overcome by a group at the National Physical Laboratory, including his former student A. Franks. Another interesting development was the fine focus X-ray tube, devised by him in collaboration with W. E. Spear. The instrument was subsequently marketed by Hilger and Watts and found application in X-ray crystallography. It was characteristic of Ehrenberg that the royalties were assigned to a fund for the development of scientific instruments.

The immediate post-war years also marked the beginning of Ehrenberg's pioneering studies on the conductivity induced in insulating solids under

electron bombardment, which eventually was to become his major concern. His 1951 paper with F. Ansbacher is still widely quoted and was one of the first detailed investigations in this field, which he and his collaborators continued to develop in subsequent years. His major work *Electronic Conduction in Semiconductors and Metals* was published in 1958 by Oxford University Press and contains the fruit of much original thought on the subject.

His appointment in 1951 to a Readership at Birkbeck College was followed in 1962 by a Chair of Experimental Physics; two years later, Ehrenberg became head of the Physics Department. Administrative responsibility thus came late in his career, but he applied to its problems the same penetrating and flexible mind that had enabled him to redirect his interests first from philosophy to physics and then successively between several fields of specialisation. After retirement in 1968 he continued to examine, to supervise research and to participate actively in colloquia. In his final years he brought to bear on the preparation of a book on *Cause, Necessity and Chance* his combined knowledge of three classical philosophy, science and languages.

Werner Ehrenberg was a remarkable man, who showed great courage in facing and overcoming the many difficulties caused by his physical disability. His humanity, enthusiasm, experimental ingenuity and physical insight were an inspiration to all, and especially to his research students, who held him in deep affection for his total devotion to the search for truth and for the generosity and imagination with which he would enter, often at a moment's notice, into a discussion of almost any problem on the enquirer's terms. His friendship was as unforgettable as it will be irreplaceable.

Reports and publications

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Physics and Ethics: The Influence of Newton on

Person to person

Three-bedroom furnished home available from September '76-Aug. '77. Similar accommodations required in Paris for 8 months, beginning Sept. '76. Please contact Dr B. Sarkar, Hospital for Sick Children, Toronto, Ontario, Canada, for further details.

Moral Philosophy. By Professor D. D. Raphael. (Inaugural Lecture, 11 March, 1975.) Pp. 17-23. 50p. Bonds and Bands. By Professor N. H. March. Pp. 25-35. (Inaugural Lecture, 29 April, 1975.) Pp. 25-35. 80p. (London: Imperial College of Science and Technology, 1975.) [261]

University of London. University College Annual Report, 1974/1975. Pp. 156. (London: University College, 1976.) [281]

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